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(54) RECONSTITUTED HUMAN ANTIBODY AGAINST HUMAN INTERLEUKIN-8

(57) A reconstituted human antibody against human interleukin-8 (IL-8), comprising (A) L chains comprising: (1) the human L-chain C region, and (2) the L-chain V region containing the human L-chain FR and the L-chain CDR of a mouse monoclonal antibody against IL-8; and (B) H chains comprising: (1) the human H-chain C region, and (2) the H-chain V region containing the human H-chain FR and the H-chain CDR of a mouse monoclonal antibody against IL-8. As the major part of the reconstituted antibody derives from a human antibody and antigenicity of the CDR is low, this antibody has a low antigenicity against the human body, thus being expected to be applicable for medical therapy.

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Description

TECHNICAL FIELD

5 The present invention relates to the complementarity determining regions (CDRs) and the variable regions (V regions) of mouse monoclonal antibody against human interleukin-8 (IL-8), to human/mouse chimeric antibody against human IL-8, as well as to a reshaped human antibody wherein the complementarity determining regions of the human light chain (L chain) variable region and the human heavy chain (H chain) variable region are substituted with the CDR of mouse monoclonal antibody against human IL-8. Moreover, the present invention provides DNAs that code for the above-mentioned antibody and its portions. The present invention also relates to a vector that contains the above-mentioned DNA, and more particularly, to an expression vector and a host transformed with said vector. Moreover, the present invention provides a process for producing reshaped human antibody against human IL-8 as well as a process for producing a chimeric antibody against human IL-8.

15 BACKGROUND ART

20 Interleukin-8 (IL-8) was discovered in the culture supernatant of monocytes stimulated with lipopolysaccharide (LPS), and is a chemokine known also as monocyte-derived neutrophil chemotactic factor (MDNCF) or neutrophil activating protein-1 (NAP-1). IL-8 is produced by various cells, acts on polymorphonuclear leukocytes and lymphocytes, and possesses activity that causes chemotaxis along its concentration gradient. In addition, not only does it induce chemotaxis in neutrophils, but it also activates neutrophilic functions such as degranulation, the release of superoxide, and the promotion of adhesion to endothelial cells.

25 In inflammatory diseases, and more specifically in respiratory diseases such as pulmonary cystic fibrosis, idiopathic pulmonary fibrosis, adult respiratory distress syndrome, sarcoidosis and empyema, as well as in skin diseases such as psoriasis, and in chronic rheumatoid arthritis, Crohn's disease and ulcerative colitis, leukocyte infiltration is observed pathologically at the inflamed site of these diseases. In addition, IL-8 is detected in test samples from patients with these diseases, suggesting that IL-8 may play a central role in inflammation. (McElvaney, N.G. et al., J. Clin. Invest., 90, 1296-1301, 1992; Lynch III, J.P. et al., Am. Rev. Respir. Dis., 145, 1433-1439, 1992; Donnelly, S.C. et al., Lancet, 341, 643-647, 1993; Car, B.D. et al., Am. J. Respir. Crit. Care Med., 149, 655-659, 1994; Antony, V.B. et al., J. Immunol., 151, 7216-7223, 1993; Takematsu, H. et al., Arch. Dermatol., 129, 74-80, 1993; Brennan, F.M. et al., Eur. J. Immunol., 20, 2141-2144, 1990; Izzo, R.S. et al., Scand. J. Gastroenterol., 28, 296-300, 1993; Izzo, R.S. et al., Am. J. Gastroenterol., 87, 1447-1452, 1992).

30 Subsequence to immunizing mice with human IL-8 as antigen, Ko, Y-C. et al. prepared the mouse monoclonal antibody WS-4 that binds to human IL-8 and inhibits the binding of human IL-8 to neutrophils as a result of that binding, namely that neutralizes the biological activity possessed by human IL-8. It has been clearly shown that the isotypes of mouse monoclonal antibody WS-4 consist of a κ-type L chain and a Cyl-type H chain (J. Immunol. Methods, 149, 227-235, 1992).

35 Known examples of antibodies against human IL-8 other than WS-4 include A.5.12.14 (Boylan, A.M. et al., J. Clin. Invest., 89, 1257-1267, 1992), the anti-Pep-1 antibody and anti-Pep-3 antibody disclosed in International Patent Application No. WO92-04372, and DM/C7 (Mulligan, M.S. et al., J. Immunol., 150, 5585-5595, 1993).

40 It was also found by administration of the mouse monoclonal antibody WS-4 into experimental models using rabbits that neutrophil infiltration is inhibited in pulmonary ischemic and reperfusion injury (Sekido, N. et al., Nature, 365, 654-657, 1993), LPS-induced dermatitis (Harada, A. et al., Internat. Immunol., 5, 681-690, 1993) and LPS- or interleukin-1 (IL-1)-induced arthritis (Akahoshi, T. et al., Lymphokine Cytokine Res., 13, 113-116, 1994).

45 A homologue of human IL-8 exists in rabbits, and is referred to as rabbit IL-8. Since it has been clearly shown that the mouse monoclonal antibody WS-4 cross-reacts with rabbit IL-8, and that the antibody inhibits binding of rabbit IL-8 to rabbit neutrophils (Harada, A. et al., Internat. Immunol., 5, 681-690, 1993), these findings suggest that anti-human IL-8 antibody would be useful as a therapeutic agent for the treatment of inflammatory diseases in humans.

50 Monoclonal antibodies originating in mammals other than humans exhibit a high degree of immunogenicity (also referred to as antigenicity) in humans. For this reason, even if mouse antibody is administered to humans, as a result of its being metabolized as a foreign substance, the half life of mouse antibody in humans is relatively short, thus preventing its anticipated effects from being adequately demonstrated. Moreover, human anti-mouse antibody that is produced in response to administered mouse antibody causes an immune response that is both uncomfortable and dangerous for the patient, examples of which include serum sickness or other allergic response. For this reason, mouse antibody cannot be administered frequently to humans.

55 In order to resolve these problems, a process for producing a humanized antibody was developed. Mouse antibody can be humanized by two methods. The simpler method involves producing a chimeric antibody in which the variable region (V region) is derived from the original mouse monoclonal antibody, and the constant region (C region) is derived from a suitable human antibody. Since the resulting chimeric antibody contains the variable region of the mouse anti-

body in its complete form, it has identical specificity to the original mouse antibody, and can be expected to bind to antigen.

Moreover, in the chimeric antibody, since the proportion of protein sequences derived from an animal other than human is substantially reduced in comparison to the original mouse antibody, it is predicted to have less immunogenicity in comparison to the original mouse antibody. Although the chimeric antibody binds well to antigen and has low immunogenicity, there is still the possibility of an immune response to the mouse variable region occurring, however (LoBuglio, A.F. et al., Proc. Natl. Acad. Sci. USA, 86, 4220-4224, 1989).

Although the second method for humanizing mouse antibody is more complexed, the latent immunogenicity of the mouse antibody is reduced considerably. In this method, only the complementarity determining region (CDR) is grafted from the variable region of mouse antibody onto the human variable region to create a reshaped human variable region. However, in order to approximate more closely the structure of the CDR of the reshaped human variable region to the structure of the original mouse antibody, there are cases in which it may be necessary to graft a portion of the protein sequence of the framework region (FR) supporting the CDR from the variable region of the mouse antibody to the human variable region.

Next, these reshaped human variable regions are linked to the human constant region. Those portions derived from non-human protein sequences consist only of the CDR and a very slight portion of the FR in the humanized antibody. CDR is composed of hyper-variable protein sequences, and these do not exhibit species specificity. For this reason, the reshaped human antibody that contains the mouse CDRs ought not to have immunogenicity stronger than that of a natural human antibody containing human CDRs.

Additional details regarding reshaped human antibodies can be found by referring to Riechmann, L. et al., Nature, 332, 323-327, 1988; Verhoeven, M. et al., Science, 239, 1534-1536, 1988; Kettleborough, C. A. et al., Protein Eng., 4, 773-783, 1991; Maeda, H. et al., Hum. Antibodies Hybridomas, 2, 124-134, 1991; Gorman, S.D. et al., Proc. Natl. Acad. Sci. USA, 88, 4181-4185, 1991; Tempest, P.R. et al., Bio/Technology, 9, 266-271, 1991; Co, M.S. et al., Proc. Natl. Acad. Sci. USA, 88, 2869-2873, 1991; Carter, P. et al., Proc. Natl. Acad. Sci. USA, 89, 4285-4289, 1992; Co, M.S. et al., J. Immunol., 148, 1149-1154, 1992; and, Sato, K. et al., Cancer Res., 53, 851-856, 1993.

DISCLOSURE OF THE INVENTION

As stated above, although reshaped human antibodies are predicted to be useful for the purpose of therapy, there are no known reshaped human antibodies against human IL-8. Moreover, there are no standard processes that can be applied universally to an arbitrary antibody for producing reshaped human antibody. Thus, various contrivances are necessary to create a reshaped human antibody that exhibits sufficient binding activity and/or neutralizing activity with respect to a specific antigen (for example, Sato, K. et al., Cancer Res., 53, 851-856, 1993). The present invention provides an antibody against human IL-8 having a low degree of immunogenicity.

The present invention provides a reshaped human antibody against human IL-8. The present invention also provides a human/mouse chimeric antibody that is useful in the production process of said reshaped human antibody. Moreover, the present invention also provides a fragment of reshaped human antibody. In addition, the present invention provides an expression system for producing chimeric antibody and reshaped human antibody and fragments thereof. Moreover, the present invention also provides a process for producing chimeric antibody against human IL-8 and fragments thereof, as well as a process for producing reshaped human antibody against human IL-8 and fragments thereof.

More specifically, the present invention provides:

- (1) an L chain V region of mouse monoclonal antibody against human IL-8; and,
- (2) an H chain V region of mouse monoclonal antibody against human IL-8.

Moreover, the present invention provides:

- (1) an L chain comprising a human L chain C region, and an L chain V region of mouse monoclonal antibody against human IL-8; and,
- (2) an H chain comprising a human H chain C region, and an H chain V region of mouse monoclonal antibody against human IL-8.

Moreover, the present invention also provides chimeric antibody against human IL-8 comprising:

- (1) L chains each comprising a human L chain C region, and an L chain V region of mouse monoclonal antibody against human IL-8; and,
- (2) H chains each comprising a human H chain C region, and an H chain V region of mouse monoclonal antibody against human IL-8.

Moreover, the present invention provides:

- (1) an L chain V region of mouse monoclonal antibody WS-4 against human IL-8; and,
- (2) an H chain V region of mouse monoclonal antibody WS-4 against human IL-8.

Moreover, the present invention also provides:

- (1) an L chain comprising a human L chain C region, and an L chain V region of mouse monoclonal antibody WS-4 against human IL-8; and,
- (2) an H chain comprising a human H chain C region, and an H chain V region of mouse monoclonal antibody WS-4 against human IL-8.

In addition, the present invention provides chimeric antibody against human IL-8 comprising:

- (1) L chains each comprising a human L chain C region, and an L chain V region of mouse monoclonal antibody WS-4 against human IL-8; and,
- (2) H chains each comprising a human H chain C region, and an H chain V region of mouse monoclonal antibody WS-4 against human IL-8.

Moreover, the present invention provides:

- (1) CDR of an L chain V region of monoclonal antibody against human IL-8; and,
- (2) CDR of an H chain V region of monoclonal antibody against human IL-8.

Moreover, the present invention also provides:

- (1) CDR of an L chain V region of mouse monoclonal antibody against human IL-8; and,
- (2) CDR of an H chain V region of mouse monoclonal antibody against human IL-8.

Moreover, the present invention also provides a reshaped human L chain V region of an antibody against human IL-8 comprising:

- (1) framework regions (FRs) of a human L chain V region; and,
- (2) CDRs of an L chain V region of mouse monoclonal antibody against human IL-8;

as well as a reshaped human H chain V region of antibody against human IL-8 comprising:

- (1) FRs of a human H chain V region; and,
- (2) CDRs of an H chain V region of mouse monoclonal antibody against human IL-8.

Moreover, the present invention provides a reshaped human L chain of antibody against human IL-8 comprising:

- (1) a human L chain C region; and,
- (2) an L chain V region comprising human L chain FRs and L chain CDRs of mouse monoclonal antibody against human IL-8;

as well as a reshaped human H chain of antibody against human IL-8 comprising:

- (1) a human H chain C region; and,
- (2) an H chain V region comprising human H chain FRs and H chain CDRs of mouse monoclonal antibody against human IL-8.

In addition, the present invention also provides reshaped human antibody against human IL-8 comprising:

(A) L chains each comprising:

- (1) a human L chain C region; and,
- (2) an L chain V region comprising FRs of a human L chain, and CDRs of an L chain of mouse monoclonal antibody against human IL-8; as well as

(B) H chains each comprising:

5 (1) a human H chain C region; and,
 (2) an H chain V region comprising FRs of a human H chain, and CDRs of an H chain of mouse monoclonal antibody against human IL-8.

More specifically, the present invention provides:

10 (1) CDRs of an L chain V region of mouse monoclonal antibody WS-4 against human IL-8 having the following sequences or a portion thereof:

CDR1: Arg Ala Ser Glu Ile Ile Tyr Ser Tyr Leu Ala
 CDR2: Asn Ala Lys Thr Leu Ala Asp
 CDR3: Gln His His Phe Gly Phe Pro Arg Thr as well as

15 (2) CDRs of an H chain V region of mouse monoclonal antibody WS-4 against human IL-8 having the following sequences or a portion thereof:

20 CDR1: Asp Tyr Tyr Leu Ser
 CDR2: Leu Ile Arg Asn Lys Ala Asn Gly Tyr Thr Arg Glu Tyr Ser Ala Ser Val Lys Gly
 CDR3: Glu Asn Tyr Arg Tyr Asp Val Glu Leu Ala Tyr

Moreover, the present invention provides a reshaped human L chain V region of antibody against human IL-8 comprising:

25 (1) framework regions (FRs) of a human L chain V region; and,
 (2) CDRs of an L chain V region of mouse monoclonal antibody WS-4 against human IL-8; as well as

30 a reshaped human H chain V region of antibody against human IL-8 comprising:

(1) FRs of a human H chain V region; and,
 (2) CDRs of an H chain V region of monoclonal antibody WS-4 against human IL-8.

35 Moreover, the present invention provides a reshaped human L chain of antibody against human IL-8 comprising:

(1) a human L chain C region; and,
 (2) an L chain V region comprising FRs of a human L chain, and CDRs of an L chain of mouse monoclonal antibody WS-4 against human IL-8; as well as

40 a reshaped human H chain of antibody against human IL-8 comprising:

(1) a human H chain C region; and,
 (2) an H chain V region comprising FRs of a human H chain, and CDRs of an H chain of monoclonal antibody WS-4 against human IL-8.

45 In addition, the present invention also provides a reshaped human antibody against human IL-8 comprising:

(A) L chains each comprising:

50 (1) a human L chain C region; and,
 (2) an L chain V region comprising FRs of a human L chain and CDRs of an L chain of mouse monoclonal antibody WS-4 against human IL-8; and

(B) H chains each comprising:

55 (1) a human H chain C region; and,
 (2) an H chain V region comprising FRs of a human H chain and CDRs of an H chain of mouse monoclonal antibody WS-4 against human IL-8.

Examples of the above-mentioned FRs of a human L chain include those having the following amino acid sequences or a portion thereof:

5 FR1: Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
 FR2: Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
 FR3: Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys
 FR4: Phe Gly Gln Gly Thr Lys Val Glu Ile Lys or,
 FR1: Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
 10 FR2: Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
 FR3: Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys
 FR4: Phe Gly Gln Gly Thr Lys Val Glu Ile Lys

15 Examples of the above-mentioned FRs of a human H chain include those having the following amino acid sequences or a portion thereof:

FR1: Glu Val Gln Leu Leu Glu Ser Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
 20 FR2: Try Val Arg Gln Ala Gln Gly Lys Gly Leu Glu Leu Val Gly
 FR3: Arg Leu Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu Ala Val Tyr Tyr Cys Ala Arg
 FR4: Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser;
 FR1: Glu Val Gln Leu Leu Glu Ser Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
 25 FR2: Trp Val Arg Gln Ala Gln Gly Lys Gly Leu Glu Trp Val Gly
 FR3: Arg Leu Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu Ala Val Tyr Tyr Cys Ala Arg
 FR4: Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser;
 30 FR1: Glu Val Gln Leu Leu Glu Ser Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
 FR2: Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Leu Val Gly
 FR3: Arg Leu Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu Ala Val Tyr Tyr Cys Ala Arg
 35 FR4: Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser;
 FR1: Glu Val Gln Leu Leu Glu Ser Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
 FR2: Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Gly
 FR3: Arg Leu Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu Ala Val Tyr Tyr Cys Ala Arg
 40 FR4: Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser;
 FR1: Glu Val Gln Leu Leu Glu Ser Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
 FR2: Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Val Gly
 FR3: Arg Leu Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu Ala Val Tyr Tyr Cys Ala Arg
 45 FR4: Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser;
 FR1: Glu Val Gln Leu Leu Glu Ser Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
 FR2: Trp Val Arg Gln Ala Pro Gly Lys Ala Leu Glu Trp Val Gly
 FR3: Arg Leu Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu Ala Val Tyr Tyr Cys Ala Arg
 50 FR4: Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser;
 FR1: Glu Val Gln Leu Leu Glu Ser Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
 FR2: Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Gly
 FR3: Arg Leu Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu Ala Val Tyr Tyr Cys Ala Arg
 55 FR4: Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser;
 FR1: Glu Val Gln Leu Leu Glu Ser Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser
 FR2: Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Gly
 FR3: Arg Phe Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu Ala Val Tyr Tyr Cys Ala Arg
 FR4: Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser; or,

FR1: Glu Val Gln Leu Leu Glu Ser Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser

FR2: Trp Val Arg Gln Ala Gln Gly Lys Gly Leu Glu Trp Val Gly

FR3: Arg Phe Thr Ile Ser Arg Glu Asp Ser Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu Ala Val Tyr Tyr Cys Ala Arg

FR4: Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

In addition, the present invention also relates to DNA that codes for polypeptide that comprises the above-mentioned various antibodies, and their fragments. The present invention also relates to a vector that contains the above-mentioned DNA, an example of which is an expression vector. Moreover, the present invention provides a host that is transformed by the above-mentioned vector.

Moreover, the present invention also provides a process for producing chimeric antibody against human IL-8, and its fragments, as well as a process for producing reshaped human antibody against human IL-8, and its fragments.

15 BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 indicates the expression vectors HEF-VL-g κ and HEF-VH-g γ 1, containing the human elongation factor-1 α (HEF-1 α) promoter/enhancer, which are useful for expression of the L chain and H chain, respectively, of the antibody of the present invention.

20 Fig. 2 is a graph indicating the results of ELISA for confirmation of the binding ability to human IL-8 of the chimeric WS-4 antibody (chL/chH) of the present invention secreted into the culture medium of COS cells.

Fig. 3 is a diagram of the construction of DNA that codes for the amino acid sequences of each of the first version "a"(RVHa) of the H chain V region of reshaped human WS-4 antibody of the present invention (A), and the first version "a"(RVLa) of the L chain V region of reshaped human WS-4 antibody (B).

25 Fig. 4 is a graph indicating the results of ELISA for comparing the binding ability to human IL-8 of the L chain V region (RVLa) and the H chain V region (RVHa) of the reshaped human WS-4 antibody of the present invention in combination with, respectively, the H chain V region of chimeric WS-4 antibody (chH) and the L chain V region of chimeric WS-4 antibody (chL) expressed in COS cells, with that of the chimeric WS-4 antibody (chL/chH) of the present invention secreted into the culture medium of COS cells.

30 Fig. 5 is a graph indicating the results of ELISA for comparing the binding ability against human IL-8 of 8 types of reshaped human WS-4 antibody containing the RVLa of the present invention (RVLa/RVHa, RVLa/RVHb, RVLa/RVHc, RVLa/RVHd, RVLa/RVHe, RVLa/RVHf, RVLa/RVHg and RVLa/RVHh) secreted into the culture medium of COS cells, with that of the chimeric WS-4 antibody (chL/chH) of the present invention secreted into the culture medium of COS cells.

35 Fig. 6 is a graph indicating the results of ELISA for comparing the binding ability to human IL-8 of 8 types of reshaped human WS-4 antibody containing the second version RVLb of the present invention (RVLb/RVHa, RVLb/RVHb, RVLb/RVHc, RVLb/RVHd, RVLb/RVHe, RVLb/RVHf, RVLb/RVHg and RVLb/RVHh) produced in the culture supernatant of COS cells, with that of the chimeric WS-4 antibody (chL/chH) of the present invention secreted into the culture medium of COS cells.

40 Fig. 7 is a graph indicating the results of ELISA for comparing the binding abilities to human IL-8 of the purified reshaped human WS-4 antibodies RVLa/RVHg and RVLb/RVHg of the present invention and the purified chimeric WS-4 antibody (chL/chH) of the present invention.

Fig. 8 is a graph indicating the results of ligand receptor binding inhibition assays for comparison of the ability to inhibit binding of IL-8 to the IL-8 receptor, of the purified reshaped human antibodies RVLa/RVHg and RVLb/RVHg of the present invention, with that of the mouse WS-4 antibody and the chimeric WS-4 antibody (chL/chH) of the present invention.

SPECIFIC MODE FOR CARRYING OUT THE INVENTION

50 Cloning of DNA Coding for Mouse V Region

In order to clone a gene that codes for the V region of mouse monoclonal antibody against human IL-8, it is necessary to prepare a hybridoma that produces mouse monoclonal antibody against human IL-8 for the acquisition of such a gene. After the extraction of mRNA from the hybridoma, the mRNA is converted into single-stranded cDNA according to known methods, followed by amplification of the target DNA using the polymerase chain reaction (PCR) to obtain the gene. An example of a source of this gene is the hybridoma WS-4, which produces mouse monoclonal antibody against human IL-8, produced by Ko, Y.C. et al. The process for preparing this hybridoma is described in J. Immunol. Methods, 149, 227-235, 1992, and is described later as Reference Example 1.

(1) Extraction of Total RNA

In order to clone the target DNA that codes for the V region of mouse monoclonal antibody against human IL-8, total RNA can be obtained by disrupting the hybridoma cells by guanidine thiocyanate treatment and performing cesium chloride density gradient centrifugation (Chirgwin, J.M. et al., *Biochemistry*, 18, 5294-5299, 1979). Furthermore, other methods that are used during the cloning of genes, such as that in which detergent treatment and phenol treatment are performed in the presence of a ribonuclease (RNase) inhibitor such as vanadium complex (Berger, S.L. et al., *Biochemistry*, 18, 5143-5149, 1979), can also be used.

(10) (2) cDNA Synthesis

Next, single-stranded cDNA complementary to mRNA can be obtained by treating the total RNA with reverse transcriptase using oligo(dT), an oligonucleotide complementary to the poly (A) tail located at the 3' end of mRNA, as primer, and the mRNA contained in the total RNA obtained in the above manner as template (Larrick, J.W. et al., *Bio/Technology*, 7, 934-938, 1989). In addition, a random primer may also be used at the same time. Furthermore, in the case that it is desired first to isolate mRNA, this may be done by applying the total RNA to a column of oligo(dT)-cellulose, to which the poly(A) tail of mRNA binds.

(11) (3) Amplification of DNA Coding for V Region by Polymerase Chain Reaction

Next, cDNA that codes for the above-mentioned V region is specifically amplified using the polymerase chain reaction (PCR). In order to amplify the kappa (κ) type L chain V region of mouse monoclonal antibody, the 11 types of oligonucleotide primers shown in SEQ ID Nos: 1 to 11 (Mouse Kappa Variable; MKV) and the oligonucleotide primer shown in SEQ ID No: 12 (Mouse Kappa Constant; MKC) are used as the 5' terminal primer and the 3' terminal primer, respectively. The above-mentioned MKV primers hybridize to the DNA sequence that codes for the mouse kappa-type L chain leader sequence, while the above-mentioned MKC primer hybridizes to the DNA sequence that codes for the mouse kappa-type L chain C region.

In order to amplify the H chain V region of mouse monoclonal antibody, the 12 types of oligonucleotide primers shown in SEQ ID Nos: 13 to 24 (Mouse Heavy Variable; MHV) and the oligonucleotide primer shown in SEQ ID No: 25 (Mouse Heavy Constant; MHC) are used as the 5' terminal primer and the 3' terminal primer, respectively. The above-mentioned MHV primers hybridize to the DNA sequence that codes for the mouse H chain leader sequence, while the above-mentioned MHC primer hybridizes to the DNA sequence that codes for the mouse H chain C region.

Furthermore, all 5' terminal primers (MKV and MHV) contain the sequence GTCGAC that provides a Sall restriction enzyme cleavage site near the 3' terminus, while both 3'-terminal primers (MKC and MHC) contain the nucleotide sequence CCCGGG that provides an XmaI restriction enzyme cleavage site near the 5' terminus. These restriction enzyme cleavage sites are used for the subcloning of target DNA fragments that code for both V regions into the respective cloning vectors. In the case that these restriction enzyme cleavage sites are also present in the target DNA sequence that codes for both V regions, other restriction enzyme cleavage sites should be used for subcloning into the respective cloning vectors.

(40) (4) Isolation of DNA Coding for V Region

Next, in order to obtain the DNA fragment that codes for the target V region of mouse monoclonal antibody, the PCR amplification products are separated and purified on a low melting-point agarose gel or by a column [PCR Product Purification kit (QIAGEN PCR Purification Spin Kit: QIAGEN); DNA purification kit (GENECLEAN II, BIO101)]. A DNA fragment is obtained that codes for the target V region of mouse monoclonal antibody by enzyme treatment of the purified amplification product with the restriction enzymes Sall and XmaI.

Further, by cleaving a suitable cloning vector, like plasmid pUC19, with the same restriction enzymes, Sall and XmaI, and enzymatically linking the above-mentioned DNA fragment to this pUC19, a plasmid is obtained which contains a DNA fragment that codes for the target V region of mouse monoclonal antibody. Determination of the sequence of the cloned DNA can be performed in accordance with any routine method, an example of which is the use of an automated DNA sequencer (Applied Biosystems). Cloning and sequence determination of the target DNA are described in detail in Examples 1 and 2.

(55) Complementarity Determining Regions (CDRs)

The present invention also provides hyper-V region or complementarity determining region (CDR) of the V region of mouse monoclonal antibody against human IL-8. V regions of both the L chain and H chain of the antibody form an antigen binding site. These regions on the L chain and the H chain have a similar basic structure. The V regions of both

chains contain four framework regions for which the sequence is relatively conserved, and these four framework regions are linked by three hyper-V regions or CDR (Kabat, E.A. et al, "Sequences of Proteins of Immunological Interest", US Dept. Health and Human Services, 1991).

The majority of the portions of the above-mentioned four framework regions (FR) have a β -sheet structure, and the three CDRs form loops. The CDRs may form a portion of the β sheet structure in some cases. The three CDRs are maintained at extremely close positions three-dimensionally by the FRs, and contribute to formation of the antigen binding site together with three paired CDRs. The present invention provides CDRs that are useful as components of humanized antibody, as well as the DNA that codes for them. These CDRs can be determined from the experimental rules of Kabat, E.A. et al. "Sequences of Proteins of Immunological Interest", by comparing V region sequences with known amino acid sequences of the V region, a detailed explanation of which is provided in Embodiment 3.

Preparation of Chimeric Antibody.

Prior to designing a reshaped human V region of antibody against human IL-8, it is necessary to confirm whether the CDRs used actually form an antigen-binding region. Chimeric antibody was prepared for this purpose. In order to prepare chimeric antibody, it is necessary to construct DNA that codes for the L chain and the H chain of chimeric antibody. The basic method for constructing both DNA involves linking the respective DNA sequences of the mouse leader sequence observed in PCR-cloned DNA and the mouse V region sequence to a DNA sequence that codes for human C region already present in a mammalian cell expression vector.

The above-mentioned human antibody C regions can be any human L chain C region and any human H chain C region, and with respect to the L chain, examples include human L chain C κ or C λ , while with respect to the H chain if IgG, examples include C γ 1, C γ 2, C γ 3 or C γ 4 (Ellison, J. et al., DNA, 1, 11-18 (1981), Takahashi, N. et al., Cell, 29, 671-679 (1982), Krawinkel, U. et al., EMBO J., 1, 403-407 (1982)), or other isotypes.

Two types of expression vectors are prepared for production of chimeric antibody, namely, an expression vector that contains DNA that codes for mouse L chain V region and human L chain C region under the control of an enhancer/promoter expression control region, and an expression vector that contains DNA that codes for mouse H chain V region and human H chain C region under the control of an enhancer/promoter type of expression control region. Next, host cells such as mammalian cells are simultaneously transformed by both of these expression vectors, and the transformed cells are cultured either in vitro or in vivo to produce chimeric antigen (e.g. WO91-16928).

Alternatively, DNA that codes for mouse L chain V region and human L chain C region and DNA that codes for mouse H chain V region and human H chain C region can be introduced into a single expression vector, host cells are transformed using said vector, and are then cultured either in vitro or in vivo to produce chimeric antibody.

The production of chimeric antibody from monoclonal antibody WS-4 is described in Embodiment 4.

cDNA that codes for mouse WS-4 κ -type L chain leader sequence and the V region is cloned using PCR, and linked to an expression vector that contains human genome DNA that codes for the human L chain C κ region. Similarly, cDNA that codes for the H chain leader sequence and V region of mouse WS-4 antibody is cloned using PCR and linked to an expression vector that contains human genome DNA that codes for human C γ 1 region.

More specifically, suitable nucleotide sequences are introduced at the 5' and 3' termini of cDNAs that code for the V regions of mouse WS-4 antibody using specially designed PCR primers so that (1) they can be easily inserted into the expression vector, and (2) they function suitably in said expression vector (for example, transcription efficiency is improved by introducing a Kozak sequence in the present invention).

Next, DNA that codes for the V region of mouse WS-4 antibody obtained by amplification by PCR using these primers is introduced into HEF expression vector (see Fig. 1) that already contains the desired human C region. These vectors are suitable for transient or stable expression of antibody genetically engineered in various mammalian cell systems.

When the antigen-binding activity of the chimeric WS-4 antibody prepared in this manner was tested, the chimeric WS-4 antibody demonstrated binding activity to human IL-8 (see Fig. 2). Thus, it was concluded that the correct mouse V region had been cloned, and the correct sequence had been determined.

50 Design of Reshaped Human WS-4 Antibody

In order to prepare a reshaped human antibody in which the CDRs of mouse monoclonal antibody are grafted onto human antibody, it is desirable that there be a high degree of homology between the amino acid sequences of the FRs of the mouse monoclonal antibody having the CDRs to be grafted, and the amino acid sequences of the FRs of the human monoclonal antibody into which the CDRs are to be grafted.

For this purpose, the human V regions to serve as the basis for designing the V regions of the reshaped human WS-4 antibody can be selected by comparing the amino acid sequences of the FRs of the mouse monoclonal antibody with the amino acid sequence of the FR of the human antibodies. More specifically, the V regions of the L and H chains of mouse WS-4 antibody were compared with all known human V regions found in the database of the National Bio-

medical Research Foundation (NBRF) using the genetic analytical software, GENETEX (Software Development Co., Ltd.).

In a comparison with known human L chain V regions, the L chain V region of mouse WS-4 antibody was found to resemble most closely that of human antibody HAU (Watanabe, S. et al., Hoppe-Seyler's Z. Physiol. Chem., 351, 1291-1295, 1970), having homology of 69.2%. On the other hand, in a comparison with known human antibody H chain V regions, the H chain V region of WS-4 antibody was found to resemble most closely that of human antibody VDH26 (Buluwela, L. et al., EMBO J., 7, 2003-2010, 1988), having homology of 71.4%.

In general, homology of the amino acid sequences of mouse V regions to the amino acid sequences of human V regions is less than the homology to amino acid sequences of mouse V regions. This indicates that the V region of mouse WS-4 antibody does not completely resemble the human V region, and at the same time, indicates that humanization of mouse WS-4 V region is the best way to solve the problem of immunogenicity in human patients.

The V region of mouse WS-4 antibody was further compared with the consensus sequence of human V region subgroup defined by Kabat, E.A. et al., (1991), Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office, to compare between V region FR. Those results are shown in Table 1.

Table 1

Homology (%) Between FR of Mouse WS-4 V Region and FR of the Consensus Sequence of the Human V Regions of Various Subgroups			
A. FR in L Chain V Region			
HSGI	HSGII	HSGIII	HSGIV
64.4	51.3	57.3	57.5
B. FR in H Chain V Region			
HSGI	HSGII	HSGIII	
46.9	40.9	62.3	

The FRs of the L chain V region of mouse WS-4 antibody most closely resembled the consensus sequence of FR of the human L chain V region subgroup I (HSGI), having homology of 64.4%. On the other hand, the FRs of the H chain V region of mouse WS-4 antibody most closely resembled the consensus sequence of human H chain V region subgroup III (HSGIII), having homology of 62.3%.

These results support the results obtained from the comparison with known human antibodies, the L chain V region of human antibody HAU belonging to human L chain V region subgroup I, and the H chain V region of human antibody VDH26 belonging to human H chain V region subgroup III. In order to design the L chain V region of reshaped human WS-4 antibody, it is probably best to use a human L chain V region belonging to subgroup I (HSGI), while in order to design the H chain V region of reshaped human WS-4 antibody, it is probably best to use the H chain V region of a human antibody belonging to subgroup III (HSGIII).

In a comparison with the L chain V region of known human antibodies, the L chain V region of mouse antibody WS-4 most closely resembled the L chain V region of human antibody REI, a member of subgroup I of human L chain V region. Thus, the FR of REI were used in designing the L chain V region of reshaped human WS-4 antibody. Within these human FR based on REI, there are differences in five amino acids (at positions 39, 71, 104, 105 and 107; see Table 2) in comparison with the human REI documented in the original literature (Palm, W. et al., Hoppe-Seyler's Z. Physiol. Chem., 356, 167-191, 1975; and, Epp, O. et al., Biochemistry, 14, 4943-4952, 1975).

The amino acid numbers shown in the table are based on the experience of Kabat, E.A. et al. (1991). The changes in the two amino acids at positions 39 and 71 were same changes caused by the amino acids present in the FR of the L chain V region of rat CAMPATH-1H antibody (Riechmann, et al., 1988). According to Kabat, et al. (1991), the changes in the other three amino acids in FR4 (positions 104, 105 and 107) are based on the J region from other human κL chains, and do not deviate from humans.

Two versions of the L chain V region of reshaped human WS-4 antibody were designed. In the first version RVL_a, FR was identical to the FR based on REI present in reshaped human CAMPATH-1H antibody (Riechmann, et al., 1988), while the CDR was identical to the CDR in the L chain V region of mouse WS-4 antibody. The second version, RVL_b, was based on RVL_a, and differed only by one amino acid at position 71 in human FR3. As defined by Chothia, C. et al., J. Mol. Biol., 196, 901-917, 1987, residue 71 is a portion of the canonical structure of the CDR1 of the L chain V region.

5 Amino acid at this position is predicted to directly affect the structure of the CDR1 loop of the L chain V region, and for this reason, it is considered to have a significant effect on antigen binding. In RVLb of the L chain V region of reshaped human WS-4 antibody, the phenylalanine at position 71 is changed to tyrosine. Table 2 shows the respective amino acid sequences of the L chain V region of mouse WS-4 antibody, the FR of the modified REI for use in reshaped human CAMPATH-1H antibody (Riechmann, et al., 1988) and the two versions of the L chain V region of reshaped human WS-4 antibody.

Table 2 Design of L Chain V Region of Reshaped Human WS-4

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	1	2	3	4
WS-4L	12345678901234567890123	45678901234	567890123456789	
REI	DIQMTQSPASLSASVG E TVTITC	RASEIIYSYLA	WYQQKQ G KSPQLLVY	
RVL _a	DIQMTQSPSSLSASVGDRV T ITC	RASEIIYSYLA	WYQQK P GKAPKLLIY	
RVL _b	-----	-----	-----	-----
	FR1	CDR1	FR2	
	5	6	7	8
WS-4L	0123456	78901234567890123456789012345678	901234567	
REI	NAKTLAD	GVSSRFSGSGSGTQFSLRISSL Q PEDFGSYYC	QHHFGFPRT	
RVL _a	NAKTLAD	GVPSRFSGSGSGT D FT T ISSL Q PEDIATYYC	QHHFGFPRT	
RVL _b	-----	-----Y-----	-----	-----
	CDR2	FR3	CDR3	
	10			
	8901234567			
WS-4L	FCGGT K LEIK			
REI	FC Q GT K <u>VEIK</u>			
RVL _a	FC Q GT K V E I K			
RVL _b	-----			
	FR4			

5 Note: FR of REI is found in reshaped human CAMPATH-1H antibody (Riechmann, et al., 1988). The five underlined amino acids in the FR of REI are amino acids that differ from the amino acid sequence of human REI. Amino acids are designated using the single letter code. Amino acid numbers are in accordance with the definition of Kabat et al.

10 15 The FR in the H chain V region of mouse WS-4 antibody most closely resemble the human H chain V region belonging to subgroup III (Table 1).

20 25 In a comparison with known human H chain V regions, the H chain V region of mouse WS-4 antibody most closely resembled the H chain V region of human antibody VDH26, a member of subgroup III of the human H chain V region, from FR1 to FR3 (Buluwela, L. et al., EMBO J., 7, 2003-2010, 1988). With respect to FR4, since the FR4 sequence of VDH26 was not reported, it was decided to use the amino acid sequence of FR4 of human antibody 4B4 belonging to subgroup III (Sanz, I. et al., J. Immunol., 142, 883-887, 1989). These human H chain V regions were used as the basis for designing the H chain V region of reshaped human WS-4 antibody.

30 35 Eight versions of the H chain V region of reshaped human WS-4 antibody were designed. In all eight versions, human FR1, FR2 and FR3 were based on FR1, FR2 and FR3 of human antibody VDH26, while FR4 was based on FR4 of human antibody 4B4. Mouse CDR was identical to the CDR of the H chain V region of mouse WS-4 antibody.

40 Tables 3 and 4 show the respective amino acid sequences of the H chain V region of mouse WS-4 antibody, the template FR1 through FR3 of human antibody VDH26, FR4 of human antibody 4B4, and the 8 versions of the H chain V region of reshaped human WS-4 antibody.

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Table 3 Design of H Chain V Region of
Reshaped Human WS-4 Antibody
(Followed by Table 4)

10	1	2	3
	123456789012345678901234567890	12345	
15	WS-4II	EVKLVESGGGLIQPGDSLRLSCVTSGFTFS	DYYLS
	VDH26	EVQELLESGGGLVQPGGSLRLSCAASGFTFS	
	RVIIa~h	EVQELLESGGGLVQPGGSLRLSCAASGFTFS	DYYLS
20		FR1	CDR1
25	4	5	6
	67890123456789	012ABC3456789012345	
	WS-4II	WVRQPPGKALEWVG	LIRNKANGYTREYSASVKG
30	VDH26	WVRQAAQGKGLELVG	
	RVIIa	WVRQAAQGKGLELVG	LIRNKANGYTREYSASVKG
35	RVIIb	-----W--	-----
	RVIIc	----P-----	-----
	RVId	----P----W--	-----
40	RVIe	----PP----W--	-----
	RVIf	----P--A--W--	-----
45	RVIg	----P----W--	-----
	RVIIh	-----W--	-----
50		FR2	CDR2

Table 4 Design of H Chain V Region of Reshaped Human WS-4
(Following on Table 3)

	7	8	9	10
10	67890123456789012ABC345678901234		567890ABC12	
15	WS-4II RFTISRDDSQSILYLQMNTLRGEDSATYYCAR		ENYRYDVELAY	
20	VDII26 RLTISREDSKNTLYLQMSSLKTEDLAVYYCAR			
25	RVIIa RLTISREDSKNTLYLQMSSLKTEDLAVYYCAR		ENYRYDVELAY	
30	RVIIb -----		-----	
35	RVIIc -----		-----	
40	RVIId -----		-----	
45	RVIIe -----		-----	
50	RVIf -----		-----	
55	RVIg -P-----		-----	
60	RVIh -P-----		-----	
		FR3		CDR3
		11		
35	34567890123			
40	WS-4II WGQGTLVTVSA			
45	4B4 WGQGTLVTVSS			
50	RVIIa-h WGQGTLVTVSS			
		FR4		

50 Note: RVHa-h indicates RVHa, RVHb, RVHc, RVHd, RVHe, RVHf, RVHg and RVHh.

55 Amino acids are designated using the single letter code. Amino acid numbers are in accordance with the definition of Kabat et al.

Preparation of DNA Coding for V Region of Reshaped Human WS-4 Antibody

Preparation of the V region of reshaped human WS-4 antibody is described in detail in Example 5.

DNAs that code for the respective first versions of the L chain and H chain V regions of reshaped human WS-4 antibody were synthesized. It was then confirmed that the entire DNA sequence of version "a" of the L chain and H chain V regions of reshaped human WS-4 antibody codes for the correct amino acid sequence by sequence determination. The sequence of version "a" of the L chain V region of reshaped human WS-4 antibody is shown in SEQ ID NO: 62, while the sequence of version "a" of the H chain V region of reshaped human WS-4 antibody is shown in SEQ ID NO: 38.

DNAs that code for other versions of V region of reshaped human WS-4 antibody were prepared using a slight variation of the publicly disclosed PCR-mutation induction method (Kammann, M. et al., Nucleic Acids Res., 17, 5404, 1989) with the first version "a" as the template. As previously described in relation to the design of the V region of the reshaped human WS-4 antibody, DNA that codes for one additional version of the L chain V region of reshaped human WS-4 antibody (version "b"), as well as DNA that code for seven additional versions of the H chain V region of reshaped human WS-4 antibody (versions "b", "c", "d", "e", "f", "g" and "h") were prepared.

These additional versions contained slight changes in a series of amino acid sequences from the first version, and these changes in the amino acid sequences were achieved by making slight changes in the DNA sequence using PCR mutation induction. A PCR primer was designed that introduces the required change in the DNA sequence. After a series of PCR reactions, the PCR product was cloned followed by sequence determination to confirm that the changes in the DNA sequence had occurred as designed. The sequence of version "b" of the L chain V region of reshaped human WS-4 antibody is shown in SEQ ID NO: 65, while the sequences of versions "b", "c", "d", "e", "f", "g" and "h" of the H chain V region of reshaped human WS-4 antibody are shown in SEQ ID Nos: 41, 44, 45, 48, 51, 54 and 55, respectively.

After confirming the DNA sequences of various versions of the V region of reshaped human WS-4 antibody by sequence determination, the DNAs that code for the V region of reshaped human WS-4 antibody were subcloned to mammalian cell expression vectors that already contain DNA that codes for the human C region. Namely, DNA that codes for the V chain L region of reshaped human WS-4 antibody was linked to a DNA sequence that codes for human L chain C region, while DNA that codes for the H chain V region of reshaped human WS-4 antibody was linked to a DNA sequence that codes for the human Cy1 region.

Next, all combinations of version "a" or "b" of the reshaped human L chain V region, and versions "a" through "h" of the H chain V region were tested for binding to human IL-8. As a result, as is shown in Fig. 7, both reshaped human antibodies containing L chain version "a" or "b" and H chain version "g" (RVLa/RVHg and RVLb/RVHg) demonstrated the ability to bind to human IL-8 to the same extent as chimeric WS-4 antibody.

Any expression system, including eukaryotic cells such as animal cells or established mammalian cells, fucus cells, yeast cells and prokaryotic cells such as bacterial cells (e.g. Escherichia coli) can be used for producing the chimeric antibody or reshaped human antibody against human IL-8 of the present invention. Preferably, however, the chimeric antibody or reshaped antibody of the present invention is expressed in mammalian cells, such as COS cells or CHO cells. In these cases, a useful, commonly used promoter can be used to express in mammalian cells. For example, it is preferable to use the human cytomegalovirus immediate early (HCMV) promoter. Examples of expression vectors that contain HCMV promoter include HCMV-VH-HC_y1 and HCMV-VL-HC_x, as well as those derived from pSV2neo (International Patent Application Publication No. WO92-19759) are also included.

In addition, examples of other promoters of genetic expression in mammalian cells that can be used in the present invention that should be used include virus promoters such as retrovirus, polioma virus, adenovirus and simian virus 40 (SV40), as well as promoters originating in mammalian cells such as human polypeptide chain elongation factor-1 α (HEF-1 α). For example, in the case of using SV40 promoter, expression can be performed by following the method of Mulligan, R.C. et al. (Nature, 277, 108-114, 1979) or in the case of using HEF-1 α promoter, expression can be performed by following the method of Mizushima, S. et al. (Nucleic Acids Res., 18, 5322, 1990).

Another specific example of a useful promoter for the present invention is HEF-1 α promoter. HEF-VH-gy1 and HEF-VL-gx (Fig. 1) are contained in an expression vector containing this promoter. DNA sequences originating in polyoma virus, adenovirus, SV40 or bovine papilloma virus (BPV) and so forth can be used as replicator points. Moreover, in order to amplify the number of genetic copies in the host cells, aminoglycoside-3'-phosphotransferase, neo-resistant gene, thymidine kinase (TK) gene, E. coli xanthin-guanine phosphoribosyl-transferase (XGPRT) gene or dihydrofolate reductase (dhfr) can be used as selection markers.

In summary, the present invention first provides an L chain V region and H chain V region of mouse monoclonal antibody against human IL-8, as well as DNA that codes for said L chain V region and DNA that codes for said H chain V region. These are useful in the preparation of human/mouse chimeric antibody and reshaped human antibody to human IL-8. An example of monoclonal antibody is WS-4. The L chain V region has the amino acid sequence shown in, for example, SEQ ID NO: 26, while the H chain V region has the amino acid sequence shown, for example, in SEQ ID NO: 27. These amino acid sequences are coded for by nucleotide sequences shown, for example, in SEQ ID Nos:

26 and 27, respectively.

The chimeric antibody against human IL-8 of the present invention comprises:

5 (1) a human L chain C region and mouse L chain V region; and,
 (2) a human H chain C region and mouse H chain V region.

The mouse L chain V region, mouse H chain V region and DNAs that code for these are as previously described. The above-mentioned human L chain C region can be any human L chain C region, examples of which include the human C_k and C_λ regions. The above-mentioned human H chain C region can be any human H chain C region, examples of which include the human C_{γ1}, C_{γ2}, C_{γ3} or C_{γ4} region (Ellison, J. et al., DNA, 1, 11-18 (1981), Takahashi, N. et al., Cell, 29, 671-679 (1982), and Krawinkel, U. et al., EMBO J., 1, 403-407 (1982)).

10 Two types of expression vectors are prepared for producing chimeric antibody. Namely, an expression vector that contains DNA that codes for the mouse L chain V region and human L chain C region under the control of an enhancer/promoter type of expression control region, and an expression vector that contains DNA that codes for the mouse H chain V region and human H chain C region under the control of an enhancer/promoter type of expression control region. Next, host cells in the manner of mammalian cells are simultaneously transformed with these expression vectors, and the transformed cells are cultured either in vitro or in vivo to produce chimeric antibody.

15 Alternatively, DNA that codes for mouse L chain V region and human L chain C region and DNA that codes for mouse H chain V region and human H chain C region can be introduced into a single expression vector, host cells are transformed using said vector, and those transformed cells are then cultured either in vitro or in vivo to produce chimeric antibody.

20 The reshaped human WS-4 antibody of the present invention comprises:

25 (A) L chains each comprising:
 (1) a human L chain C region; and,
 (2) an L chain V region comprising a human L chain FRs, and an L chain CDRs of mouse monoclonal antibody WS-4 against human IL-8, as well as

30 (B) H chains each comprising:
 (1) a human H chain C region; and,
 (2) an H chain V region comprising a human H chain FRs, and H chain CDRs of mouse monoclonal antibody WS-4 against human IL-8.

35 In a preferable mode of the present invention, the above-mentioned L chain CDR is within the amino acid sequence shown in SEQ ID NO: 26, with the extents of said amino acid sequence being defined in Table 5; the above-mentioned H chain CDR is within the amino acid sequence shown in SEQ ID NO: 27, with the extents of said amino acid sequence being defined in Table 5; the above-mentioned human L chain FR is derived from RE1; the above-mentioned human H chain FR1, FR2 and FR3 are derived from VDH26, and FR4 is derived from 4B4; the above-mentioned human L chain C region is the human C_k region; and, the above-mentioned human H chain C region is the human C_{γ1} region. In addition, the above-mentioned human H chain C region may be the human C_{γ4} region, or a radioisotope may be bound instead of the above-mentioned human L chain C region and/or human H chain C region.

40 It is preferable to substitute a portion of the amino acid sequence of the above-mentioned human FR to prepare reshaped human antibody that has sufficient activity with respect to a specific antigen.

45 In a preferable mode of the present invention, the L chain V region has the amino acid sequence shown as RVL_a or RVL_b in Table 2, while the H chain V region has the amino acid sequence shown as RVH_a, RVH_b, RVH_c, RVH_d, RVH_e, RVH_f, RVH_g or RVH_h in Tables 3 and 4. Moreover, the amino acid at position 41 in the H chain V region FR2 should be proline, the amino acid at said position 47 should be tryptophan, and/or the amino acid at position 67 of said 50 FR3 should be phenylalanine, and those having the amino acid sequences shown as RVH_b, RVH_d, RVH_e, RVH_f, RVH_g or RVH_h are more preferable. That in which RVH_g is present as the H chain V region is the most preferable.

55 Two types of expression vectors are prepared for production of reshaped antibody. Namely, an expression vector that contains DNA that codes for the previously defined reshaped human L chain under control by an enhancer/promoter type of expression control region, as well as another expression vector that contains DNA that codes for the previously defined reshaped human H chain under control by an enhancer/promoter type of expression control region, are prepared. Next, host cells such as mammalian cells are simultaneously transformed by these expression vectors, and the transformed cells are cultured either in vitro or in vivo to produce reshaped human antibody.

Alternatively, DNA that codes for reshaped human L chain and DNA that codes for reshaped human H chain are introduced into a single expression vector, host cells are transformed using said vector, and those transformed cells are

then cultured either in vitro or in vivo to produce the target reshaped human antibody.

The chimeric antibody or reshaped human antibody produced in this manner can be isolated and purified in accordance with routine methods such as protein A affinity chromatography, ion exchange chromatography or gel filtration.

5 The chimeric L chain or reshaped human L chain of the present invention can be used to prepare complete antibody by combining with an H chain. Similarly, the chimeric H chain or reshaped human H chain of the present invention can be used to prepare complete antibody by combining with an L chain.

The mouse L chain V region, reshaped human L chain V region, mouse H chain V region and reshaped human H chain V region are inherently regions that bind to antigen in the form of human IL-8. They are considered to be useful as pharmaceuticals, diagnostic drugs and so forth either alone or in the form of fused protein with other proteins.

10 In addition, the L chain V region CDR and H chain V region CDR of the present invention are also inherently portions that bind to antigen in the form of human IL-8. These are considered to be useful as pharmaceuticals, diagnostic drugs and so forth either alone or in the form of fused protein with other proteins.

15 The DNA that codes for mouse L chain V region of the present invention is useful for preparing DNA that codes for chimeric L chain, or DNA that codes for reshaped human L chain. Similarly, the DNA that codes for mouse H chain V region is useful for preparing DNA that codes for chimeric H chain or DNA that codes for reshaped human H chain. In addition, the DNA that codes for the L chain V region CDR of the present invention is useful for preparing DNA that codes for reshaped human L chain V region, or DNA that codes for reshaped human L chain.

20 Similarly, the DNA that codes for the H chain V region CDR of the present invention is useful for preparing DNA that codes for reshaped human H chain V region, and DNA that codes for reshaped human H chain. Moreover, reshaped human antibody F(ab')₂, Fab or Fv, or single chain Fv that couples both Fv of the H chain and L chain, can be produced in a suitable host and used for the purposes described above (see, for example, Bird, R.E. et al., TIBTECH, 9, 132-137, 1991).

25 Single chain Fv is composed by linking the H chain V region and L chain V region of reshaped human antibody to human IL-8. In this single chain Fv, the H chain V region and L chain V region are linked by a linker, and preferably a peptide linker (Huston, J.S. et al., Proc. Natl. Acad. Sci. USA, 85, 5879-5883, 1988).

30 The H chain V region and L chain V region of this single chain Fv may be either of the above-mentioned H chain and L chain V regions of reshaped human antibody. Specific examples of these include the H chain V regions composed of the amino acid sequences described in SEQ ID NOs: 38, 41, 44, 45, 48, 51 and 54, and single chain Fv containing an L chain V region composed of the amino acid sequences described in SEQ ID NO: 62 or 65 (see WO88-01649).

35 These V regions are preferably linked by a peptide linker. Examples of peptide linkers that are used include any arbitrary single chain peptide composed of, for example 12-19 residues (see WO88-09344).

DNA that codes for single chain Fv is obtained by using DNA that codes for the H chain or H chain V region and DNA that codes for the L chain or L chain V region of the above-mentioned reshaped human antibody as template, 40 amplifying the portion of DNA that codes for those amino acid sequences that are desired using a primer pair that defines both ends by PCR, and amplifying by combining a primer pair that defines DNA that codes for a polypeptide linker along with both its ends so as to respectively link the H and L chains.

In addition, once the DNA that codes for single chain Fv are prepared, an expression vector that contains them along with a host that is transformed by said expression vector can be obtained in accordance with routine methods. In addition, single chain Fv can be obtained in accordance with routine methods by using that host.

45 In comparison with antibody molecules, single chain Fv exhibit better permeability into tissue, and are expected to be used in imaging by labelling with a radioisotope, and as a therapeutic agent having similar functions to reshaped human antibody.

ELISA (Enzyme-linked immunosorbent assay), EIA (Enzyme immunoassay), RIA (radioimmunoassay) or fluorescent antibody techniques can be used to confirm the binding activity of the chimeric antibody, reshaped human antibody and its F(ab')₂, Fab, Fv or single chain Fv against IL-8 of the present invention. For example, in the case of using enzyme immunoassay with chimeric antibody and reshaped human antibody, human IL-8 is added to a plate coated with anti-human IL-8 polyclonal antibody, a culture supernatant or purified sample of cells that produce chimeric antibody or reshaped human antibody against human IL-8 is added, and a suitable secondary antibody is added that is labeled with an enzyme such as alkaline phosphatase. After incubating and washing the plate, an enzyme substrate such as p-nitrophenylphosphate is added followed by measurement of absorbance to evaluate the antigen binding activity.

50 The IL-8 binding inhibitory activity to IL-8 receptors of the chimeric antibody, reshaped human antibody, and its F(ab')₂, Fab, Fv or single chain Fv against human IL-8 is evaluated by an ordinary ligand receptor binding inhibition assay. For example, in order to assay the inhibition of binding of IL-8 to IL-8 receptors on neutrophils, after separating neutrophils obtained from heparinized blood by centrifugation or other means, a cell suspension is prepared having a suitable number of cells that can be used in the above-mentioned assay.

55 A solution containing IL-8 suitably labeled with ¹²⁵I and so forth and non-labeled IL-8 is mixed with a solution containing the antibody of the present invention or its fragments prepared at a suitable concentration, followed by the addition of a solution containing a suitable ligand.

tion of this mixture to the above-mentioned neutrophil suspension. After a certain period of time, the neutrophils are separated, and the labeled activity on the neutrophils is assayed.

Routine known methods, such as the method described in Grob, P.M. et al., J. Biol. Chem., 265, 8311-8316, 1990, can be used for evaluation of the inhibition of neutrophil chemotaxis by the antibody or its fragments of the present invention.

In the case of using a commercially available chemotaxis chamber, after diluting the antibody or its fragments of the present invention with a suitable culture medium, IL-8 is added to the chamber followed by the addition of the diluted antibody or fragments. Next, the prepared neutrophil suspension is added to the chamber and allowed to stand for a certain period of time. Since migrating neutrophils adhere to the filter installed in the chamber, the number of such neutrophils may be measured by ordinary methods such as staining or fluorescent antibody methods. In addition, measurement may also be performed by microscopic evaluation using a microscope or by automated measurement using a machine.

After sterilizing by filtration using a membrane filter, the chimeric antibody, reshaped human antibody and its F(ab')₂, Fab, Fv or single chain Fv fragment against human IL-8 of the present invention can be administered as a pharmaceutical therapeutic agent preferably parenterally, by for example intravenous injection, intramuscular injection, intra-peritoneal injection or subcutaneous injection, or transtracheally, by for example using a nebulizer. Although varying according to the age and symptoms of the patient, the normal dose in humans is 1-1000 mg/body, for which divided doses of 1-10 mg/kg/week can be selected.

After evaluating their purified binding activity, the chimeric antibody, reshaped human antibody and its F(ab')₂, Fab, Fv or single chain Fv fragment against human IL-8 of the present invention can be prepared into a pharmaceutical therapeutic agent by methods routinely used for making preparations of physiologically active proteins. For example, a preparation for injection consists of dissolving refined chimeric antibody, reshaped human antibody or its F(ab')₂, Fab, Fv or single chain Fv fragment against human IL-8 in a solvent such as physiological saline or buffer, followed by the addition of an anti-adsorption agent such as Tween 80, gelatin or human serum albumin (HSA). Alternatively, this preparation may also be freeze-dried for dissolution and reconstitution prior to use. Examples of vehicles that can be used for freeze-drying include sugar-alcohols or sugars such as mannitol and glucose.

EXAMPLES

Although the following provides a detailed explanation of the present invention through its embodiments described below, the scope of the present invention is not limited by these examples.

Example 1: Cloning of DNA Coding for the V Region of Mouse Monoclonal Antibody against Human IL-8

DNA that codes for the variable region of mouse monoclonal antibody against human IL-8 was cloned in the manner described below.

1. Preparation of Total RNA

Total RNA was prepared from hybridoma WS-4 by modifying the cesium chloride density gradient centrifugation method of Chirgwin, J.M. et al. described in Biochemistry, 18, 5294-5299, 1979.

Namely, 1×10^7 hybridoma WS-4 cells were completely homogenized in 25 ml of 4 M guanidine thiocyanate (Fluka). The homogenate was layered over a 5.7 M cesium chloride solution in a centrifuge tube followed by precipitation of the RNA by centrifuging for 14 hours at 20°C at 31,000 rpm in a Beckman SW40 rôtor.

The RNA precipitate was washed with 80% ethanol and then dissolved in 200 μ l of 20 mM Tris-HCl (pH 7.5) containing 10 mM EDTA and 0.5% sodium N-laurylsarcosinate. After adding Protease (Boehringer) to a concentration of 0.5 mg/ml, the resulting mixture was incubated in a water bath for 30 minutes at 37°C. The mixture was extracted with phenol and chloroform and the RNA was precipitated with ethanol. Next, the RNA precipitate was dissolved in 200 μ l of 10 mM Tris-HCl (pH 7.5) containing 1 mM EDTA.

2. Extraction of Messenger RNA (mRNA)

In order to extract mRNA coding for the H chain of mouse monoclonal antibody WS-4, poly(A)-positive mRNA was extracted from the total RNA obtained step 1 above using the Fast Track mRNA Isolation Kit Version 3.2 (Invitrogen) and following the procedure described in the manufacturer's instructions.

3. Synthesis of Single Stranded cDNA

Single stranded cDNA was synthesized from approximately 40 ng of the mRNA obtained in step 2 above using the

cDNA Cycle Kit (Invitrogen) and following the procedure described in the instructions. The resultant product was then used to amplify cDNA that codes for mouse H chain V region. Furthermore, in order to amplify cDNA that codes for mouse L chain V region, single stranded cDNA was synthesized from approximately 10 µg of the above-mentioned total RNA.

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4. Amplification of Gene Coding for Antibody Variable Region by PCR

(1) Amplification of cDNA Coding for Mouse H Chain V Region

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MHV (mouse heavy variable) primers 1 to 12 shown in SEQ ID NOs: 13 to 24 and MHC (mouse heavy constant) primer shown in SEQ ID NO: 25 (Jones, S.T. et al., Bio/Technology, 9, 88-89, 1991) were used for the PCR primers. 100 µl of PCR solution containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.1 mM dNTPs (dATP, dGTP, dCTP, dTTP), 1.5 mM MgCl₂, 0.001% (w/v) gelatin, 5 units of DNA polymerase AmpliTaq (Perkin Elmer Cetus), 0.25 µM of one of the MHV primers shown in SEQ ID NOs: 13 to 24, 75 µM of the MCH primer shown in SEQ ID NO: 25, and 1.5 µl of the single stranded cDNA solution obtained in step 3 above. PCR solutions were prepared for each of the MHV primers 1-12. After covering each solution with 50 µl of mineral oil, it was heated in the order of 3 minutes at the initial temperature of 94°C, followed by a cycle of 1 minute at 94°C, 1 minute at 55°C and 1 minute at 72°C. After repeating this heating cycle 30 times, the reaction mixture was further incubated for 10 minutes at 72°C.

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(2) Amplification of cDNA Coding for Mouse L Chain V Region

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MKV (mouse kappa variable) primers 1 to 11 shown in SEQ ID NOs: 1 to 11 and MKC (mouse kappa constant) primer shown in SEQ ID NO: 12 (Jones, S.T. et al., Bio/Technology, 9, 88-89, 1991) were used for the PCR primers.

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Amplification of cDNA was performed from 2.0 µl of the single stranded cDNA obtained in step 3 above using the same method as that described for amplification of H chain V region gene in step 4 part (1) above with the exception that amplification was performed using 0.25 µM each of the MKV primer mixtures and 3.0 µM of MCK primer.

5. Purification and Fragmentation of PCR Product

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The respective DNA fragments of the H chain V region and L chain V region amplified by PCR as described above were separated by agarose gel electrophoresis using 1.5% low melting point agarose (Sigma). Agarose pieces containing an H chain DNA fragment approximately 450 bp in length and an L chain DNA fragment approximately 400 bp in length were separately cut out and melted for 5 minutes at 65°C followed by the addition of an equal volume of 20 mM Tris-HCl (pH 7.5) containing 2 mM EDTA and 300 mM NaCl.

35

This mixture was extracted by phenol and chloroform, the DNA fragments were recovered by ethanol precipitation, and dissolved in 10 mM Tris-HCl (pH 7.5) containing 1 mM EDTA. Next, the fragments were digested for 3 hours at 37°C using 5 units of restriction enzyme XmaI (New England BioLabs) in 10 mM Tris-HCl (pH 7.9) containing 10 mM MgCl₂ and 1 mM dithiothreitol. Next, the DNA fragments were digested for 2 hours at 37°C with 40 units of restriction enzyme Sall (Takara Shuzo), and the resulting DNA fragments were separated by agarose gel electrophoresis using 1.5% low melting point agarose (Sigma).

40

The agarose pieces containing DNA fragments were cut out and melted for 5 minutes at 65°C followed by the addition of an equal volume of 20 mM Tris-HCl (pH 7.5) containing 2 mM EDTA and 300 mM NaCl. This mixture was then extracted from phenol and chloroform, the DNA fragments were recovered by ethanol precipitation and dissolved in 10 mM Tris-HCl (pH 7.5) containing 1 mM EDTA.

45

Thus, a DNA fragment containing a gene that codes for mouse κ-type L chain V region, and a DNA fragment containing a gene that codes for mouse H chain V region were respectively obtained. The above-mentioned DNA fragments both have an Sall attachment site at their 5' terminus, and an XmaI attachment site at their 3' terminus.

6. Linkage and Transformation

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Approximately 0.3 µg of the Sall-XmaI DNA fragment containing gene that codes for mouse kappa-type L chain V region prepared in the manner described above were mixed with approximately 0.1 µg of pUC19 vector (Takara Shuzo), prepared by digesting with Sall, XmaI and alkaline phosphatase of *Escherichia coli* (BAP; Takara Shuzo), for 4 hours at 16°C in a buffered reaction mixture containing 1 unit of T4 DNA ligase (Gibco BRL) and added supplemented buffer to link.

55

Next, 5 µl of the above-mentioned linkage mixture were added to 50 µl of competent cells of *E. coli* DH5α (GIBCO BRL) after which the cells were allowed to stand for 30 minutes on ice, for 1 minute at 42°C and again for 1 minute on ice. Next, 400 µl of 2 × YT medium (Molecular Cloning: A Laboratory Manual, Sambrook, et al., Cold Spring Harbor Laboratory Press, 1989) were added. After incubating for 1 hour at 37°C, the *E. coli* was spread onto 2 × YT agar medium (Molecular Cloning: A Laboratory Manual, Sambrook, et al., Cold Spring Harbor Laboratory Press, 1989) containing 50

μg/ml of ampicillin (Meiji Seika) followed by incubation overnight at 37°C to obtain the *E. coli* transformant.

Subsequently, 50 μg of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside, Takara Shuzo) were applied as selection marker at this time.

This transformant was incubated overnight at 37°C in 10 ml of 2 × YT medium containing 50 μg/ml of ampicillin, and plasmid DNA was prepared from this culture using the QIAGEN Plasmid Mini Kit (QIAGEN) and following the procedure described in the instructions.

The plasmid containing gene that codes for mouse κ-type L chain V region originating in hybridoma WS-4 obtained in this manner was named pUC-WS4-VL.

A plasmid containing gene that codes for mouse H chain V region derived from hybridoma WS-4 was prepared from Sall-XmaI DNA fragments by following the same method as described above with the exception of using JM109 for the *E. coli* competent cells. The resulting plasmid was named pUC-WS4-VH.

Example 2: Determination of DNA Nucleotide Sequence

The nucleotide sequence of the cDNA coding region in the above-mentioned plasmids was determined using M13 Primer RV and M13 Primer M4 (both Takara Shuzo) as sequence primers, an automated DNA sequencer (Applied Biosystems Inc.) and the Taq Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems Inc.) and following the protocol specified by the manufacturers. The nucleotide sequence of the gene that codes for the L chain V region of mouse WS-4 antibody contained in plasmid pUC-WS4-VL is shown in SEQ ID NO: 26. In addition, the nucleotide sequence of the gene that codes for the H chain V region of mouse WS-4 antibody contained in plasmid pUC-WS4-VH is shown in SEQ ID NO: 27.

Example 3: Determination of CDR

The basic structure of the V regions of the L and H chains has mutual similarities, each having four framework regions linked by three hyper variable regions, namely complementarity determining regions (CDR). Although the amino acid sequence of the framework region is relatively well preserved, the variability of the amino acid sequence of the CDR regions is extremely high (Kabat, E.A. et al., "Sequences of Proteins of Immunological Interest", US Dept. of Health and Human Services, 1991).

On the basis of this fact, the CDR were determined as shown in Table 5 by investigating their homology by attempting to match the amino acid sequence of the variable region of mouse monoclonal antibody to human IL-8 with the database of amino acid sequences of antibodies prepared by Kabat, et al.

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Table 5

CDR in the L Chain V Region and H Chain V Region of Mouse WS-4 Antibody				
Plasmid	Sequence Number	CDR1	CDR2	CDR3
pUC-WS4-VL	26	24-34	50-56	89-97
pUC-WS4-VH	27	31-35	50-68	101-111

45

Example 4: Confirmation of Expression of Cloned cDNA (Preparation of Chimeric WS-4 Antibody)

Preparation of Expression Vector

In order to prepare a vector that expresses chimeric WS-4 antibody, cDNA clones pUC-WS4-VL and pUC-WS4-VH, which code for the L chain and H chain V regions of mouse WS-4, respectively, were modified by PCR. These were then introduced into HEF expression vector (refer to that previously described, WO92-19759 and Fig. 1).

The backward primer (SEQ ID NO: 28) for the L chain V region and the backward primer (SEQ ID NO: 29) for the H chain V region were respectively hybridized to DNA that codes for the start of the leader sequence of the V region, and designed to have a Kozak consensus sequence (Kozak, M. et al., J. Mol. Biol., 196, 947-950, 1987) and a HindIII restriction site. The forward primer (SEQ ID NO: 30) for the L chain V region and the forward primer (SEQ ID NO: 31) for the H chain V region were hybridized to a DNA sequence that codes for the terminal of the J chain, and designed to add a splice donor sequence and BamHI restriction site.

100 μl of PCR reaction mixture containing 20 mM Tris-HCl (pH 8.2), 10 mM KCl, 6 mM (NH₄)₂SO₄, 1% Triton X-

100, 100 μ M dNTPs, 1.5 mM MgCl₂, 100 pmoles of each primer, 100 ng of template DNA (pUC-VL or pUC-VH) and 2.5 U of AmpliTaq enzyme, were covered with 50 μ l of mineral oil. After initially denaturing for 3 minutes at 94°C, a heating cycle consisting of 1 minute at 94°C, 1 minute at 55°C and 1 minute at 72°C was repeated 30 times followed by final incubation for 10 minutes at 72°C.

5 The PCR product was purified using 1.5% low melting point agarose gel followed by digestion with HindIII and BamHI. The L chain V region was cloned into HEF expression vector HEF-VL-g_k, while the H chain V region was cloned into HEF expression vector HEF-VH-g_{y1}. After determining the DNA sequences, plasmids containing the DNA fragment having the correct DNA sequence were named HEF-chWS4L-g_k and HEF-chWS4H-g_{y1} respectively.

10 Transfection into COS Cells

In order to observe the transient expression of chimeric WS-4 antibody, the above-mentioned expression vectors were tested in COS cells. HEF-chWS4L-g_k and HEF-chWS4H-g_{y1} were simultaneously transfected into COS cells by electroporation using the Gene Pulser system (BioRad). Each DNA (10 μ g) was added to 0.8 ml of aliquot containing 15 $\times 10^7$ cells/ml in PBS, and then pulsed at 1.5 kV with a capacitance of 25 μ F.

After allowing a recovery period of 10 minutes at room temperature, the electroporated cells were suspended in 1 ml of DMEM culture medium (GIBCO) containing 5% γ -globulin-free fetal bovine serum placed in a tissue culture dish. After incubating for 96 hours, the culture medium was collected, cell debris were removed by centrifugation, and the supernatant was then filtered with a disk filter having a pore diameter of 0.45 μ m (Gelman Science).

20 ELISA

ELISA plates for measurement of antigen binding and antibody concentration were prepared as described below. The ELISA plates for measurement of antigen binding activity were prepared in the following manner. After forming a solid layer in each well of a 96-well plate (Nunc) with 100 μ l of goat anti-human IL-8 polyclonal antibody (R & D Systems) dissolved in a solid layer of buffer at a concentration of 2 μ g/ml (0.1 M sodium bicarbonate, 0.02% sodium azide), and blocking with 200 μ l of dilution buffer (50 mM Tris-HCl (pH 7.2), 1% bovine serum albumin (BSA), 1 mM MgCl₂, 0.15 M NaCl, 0.05% Tween 20, and 0.02% sodium azide), 100 μ l of recombinant human IL-8 (Amersham) (5 ng/ml) was added.

30 A purified sample of chimeric antibody or culture supernatant of COS cells that expressed these was serially diluted and added to each well. Next, 100 μ l of alkaline phosphatase-labeled goat anti-human IgG antibody (TAGO) (1 μ g/ml) were added. After incubation and washing, substrate solution (1 mg/ml p-nitrophenyl-phosphate) was added followed by measurement of absorbance at 405 nm.

35 For measurement of antibody concentration, after forming a solid layer in the wells of a 96-well plate with 100 μ l of goat anti-human IgG antibody (TAGO) at a concentration of 1 μ g/ml and blocking, a purified sample of chimeric antibody or culture medium of COS cells that expressed these was serially diluted and added to each well. Next, 100 μ l of alkaline phosphatase-labeled goat anti-human IgG antibody (TAGO) (1 μ g/ml) was added. After incubation and washing, substrate solution (1 mg/ml p-nitrophenylphosphate) was added and absorbance was measured at 405 nm.

40 As a result, since the chimeric antibody WS-4 showed specific binding to IL-8, it was considered that this chimeric antibody has the correct structure of the V region of mouse monoclonal antibody WS-4 (see Fig. 2).

Furthermore, the Escherichia coli having above-mentioned plasmid HEF-chWS4L-g_k was deposited as Escherichia coli DH5 α (HEF-chWS4L-g_k), and the Escherichia coli having the above-mentioned plasmid HEF-chWS4H-g_{y1} was deposited as Escherichia coli JM109 (HEF-chWS4H-g_{y1}) at the Bioengineering Industrial Technology Research Institute of the Agency of Industrial Science and Technology (1-1-3 Higashi, Tsukuba, Ibaraki, Japan) on 45 July 12, 1994 under the respective names FERM BP-4739 and FERM BP-4740 in accordance the provisions of the Budapest Convention.

Example 5: Preparation of Reshaped Human WS-4 Antibody

50 Preparation of the H Chain V Region of Reshaped Human WS-4 Antibody

DNA that codes for the H chain V region of reshaped human WS-4 antibody was designed in the manner described below. Complete DNA that codes for the H chain V region of reshaped human WS-4 antibody was designed so that known DNA sequences that respectively code for FR1 through FR3 of human antibody VDH26 and FR4 of human antibody 4B4 are linked to the DNA sequence that codes for the CDR of the H chain V region of mouse WS-4 antibody.

55 Next, a HindIII recognition site/Kozak consensus sequence and BamHI recognition site/splice donor sequence were respectively added to the 5' and 3' sides of this DNA sequence, followed by introduction into an HEF expression vector. The DNA sequence designed in this manner was then divided into four approximately equal oligonucleotides after which the secondary structure of those oligonucleotides for which there is the possibility of obstructing the assem-

bly of these oligonucleotides were analyzed by computer.

The four oligonucleotide sequences are shown in SEQ ID NOs: 32 to 35. These oligonucleotides have lengths of 113 to 143 bases, and adjacent oligonucleotides have an overlap region mutually consisting of 20 bases. HF1 (SEQ ID NO: 32) and HF3 (SEQ ID NO: 34) of these four oligonucleotides have a sense DNA sequence, while the other HF2 (SEQ ID NO: 33) and HF4 (SEQ ID NO: 35) have an antisense DNA sequence. These oligonucleotides were synthesized by an automated DNA synthesizer (Applied Biosystems).

In addition, the method of assembly of these four oligonucleotides by PCR is illustrated in Fig. 3. Approximately 100 ng each of HF1 and HF2 as well as HF3 and HF4 were combined and added to a PCR reaction mixture having a final volume of 98 μ l and containing 2.5 U of Pfu DNA polymerase. After initially denaturing for 3 minutes at 94°C, the solutions were incubated for 2 cycles each cycle consisting of incubation for 2 minutes at 94°C, 2 minutes at 55°C and 2 minutes at 72°C.

After mutually replacing half the volume of the PCR reaction solutions, incubation was continued for an additional two cycles. After adding 100 pmoles each of RVH5' primer (SEQ ID NO: 36) and RVH3' primer (SEQ ID NO: 37) as external primers, the PCR reaction solutions were covered with 50 μ l of mineral oil. After initially denaturing for 3 minutes at 94°C, the reaction solutions were incubated for 45 cycles of 1 minute at 94°C, 1 minute at 55°C and 1 minute at 72°C, followed finally by incubation for 10 minutes at 72°C.

A DNA fragment containing approximately 450 base pairs was purified on a 1.5% low melting point agarose gel, digested with HindIII and BamHI and cloned into HEF expression vector HEF-VH-g γ 1 (Fig. 1). After determining the DNA sequence using EF-1 primer (SEQ ID NO: 66) and HIP primer (SEQ ID NO: 67), the plasmid that contained a DNA fragment that codes for the correct amino acid sequence of the H chain V region was named HEF-RVHa-g γ 1. The amino acid sequence and nucleotide sequence of the H chain V region contained in this plasmid HEF-RVHa-g γ 1 are shown in SEQ ID NO: 38.

Each of the versions "b", "c", "d", "e", "f", "g" and "h" of the H chain V region of reshaped human WS-4 antibody was prepared in the manner described below.

Version "b" (RVHb) was amplified by PCR using mutagen primers LTW1 (SEQ ID NO: 39) and LTW2 (SEQ ID NO: 40), designed so that leucine at position 47 was replaced by tryptophan, RVH5' (SEQ ID NO: 36) and RVH3' (SEQ ID NO: 37) for the primers that define both ends, and plasmid HEF-RVHa-g γ 1 as the template DNA to obtain plasmid HEF-RVHb-g γ 1. The amino acid sequence and nucleotide sequence of the H chain V region contained in this plasmid HEF-RVHb-g γ 1 are shown in SEQ ID NO: 41.

Version "c" was amplified by PCR using mutagen primers QTP1 (SEQ ID NO: 42) and QTP2 (SEQ ID NO: 43), designed so that glutamic acid at position 41 was replaced by proline, and plasmid HEF-RVHa-g γ 1 as the template DNA to obtain plasmid HEF-RVHc-g γ 1. The amino acid sequence and nucleotide sequence of the H chain V region contained in this plasmid HEF-RVHc-g γ 1 are shown in SEQ ID NO: 44.

Version "d" was amplified by PCR using mutagen primers QTP1 and QTP2 and plasmid HEF-RVHb-g γ 1 as the template DNA to obtain plasmid HEF-RVHd-g γ 1. The amino acid sequence and nucleotide sequence of the H chain V region contained in this plasmid HEF-RVHd-g γ 1 are shown in SEQ ID NO: 45.

Version "e" was amplified by using mutagen primers ATP1 (SEQ ID NO: 46) and ATP2 (SEQ ID NO: 47), designed so that alanine at position 40 was replaced by proline, and plasmid HEF-RVHd-g γ 1 as the template DNA to obtain plasmid HEF-RVHe-g γ 1. The amino acid sequence and nucleotide sequence of the H chain V region contained in this plasmid HEF-RVHe-g γ 1 are shown in SEQ ID NO: 48.

Version "f" was amplified using mutagen primers GTA1 (SEQ ID NO: 49) and GTA2 (SEQ ID NO: 50), designed so that glycine at position 44 was replaced by alanine, and plasmid HEF-RVHd-g γ 1 for the template DNA to obtain plasmid HEF-RVHf-g γ 1. The amino acid sequence and nucleotide sequence of the H chain V region contained in this plasmid HEF-RVHf-g γ 1 are shown in SEQ ID NO: 51.

Version "g" was amplified using mutagen primers LTF1 (SEQ ID NO: 52) and LTF2 (SEQ ID NO: 53), designed so that leucine at position 67 was replaced by phenylalanine, and plasmid HEF-RVHd-g γ 1 as the template DNA to obtain plasmid HEF-RVHg-g γ 1. The amino acid sequence and nucleotide sequence of the H chain V region contained in this plasmid HEF-RVHg-g γ 1 are shown in SEQ ID NO: 54.

Version "h" was amplified using mutagen primers LTF1 and LTF2, and plasmid HEF-RVHb-g γ 1 as the template DNA to obtain plasmid HEF-RVHh-g γ 1. The amino acid sequence and nucleotide sequence of the H chain V region contained in this plasmid HEF-RVHh-g γ 1 are shown in SEQ ID NO: 55.

Preparation of L Chain V Region of Reshaped Human WS-4 Antibody

DNA that codes for the L chain V region of reshaped human WS-4 antibody was designed in the manner described below. Complete DNA that codes for the L chain V region of reshaped human WS-4 antibody was designed so that a DNA sequence that codes for the FR of human antibody RE1 is linked to the DNA sequence that codes for the CDR of the L chain V region of mouse WS-4 antibody.

Next, a HindIII recognition site/Kozak consensus sequence and BamHI recognition site/splice donor sequence

were respectively added to the 5' and 3' sides of this DNA sequence so as to enable it to be introduced into an HEF expression vector. The DNA sequence designed in this manner was then divided into four approximately equal oligonucleotides after which the secondary structure of those oligonucleotides for which there is the possibility of obstructing the assembly of these oligonucleotides were analyzed by computer.

5 The four oligonucleotide sequences are shown in SEQ ID NOs: 56 to 59. These oligonucleotides have lengths of 106 to 124 bases, and adjacent oligonucleotides have an overlap region mutually consisting of 19 to 23 bases. LF1 (SEQ ID NO: 56) and LF3 (SEQ ID NO: 58) of these four oligonucleotides have a sense DNA sequence, while the other LF2 (SEQ ID NO: 57) and LF4 (SEQ ID NO: 59) have an antisense DNA sequence. These oligonucleotides were synthesized using the same method as that employed for the above-mentioned HF1 through HF4.

10 For assembly, after initially denaturing 98 μ l of a PCR mixture containing 100 ng of each of the four types of the nucleotides and 5 U of Ampli Tag for 3 minutes at 94°C, the mixture was incubated for 2 cycles, each cycle consisting of incubation for 2 minutes at 94°C, 2 minutes at 55°C and 2 minutes at 72°C. After adding 100 pmoles each of RVL5' primer (SEQ ID NO: 60) and RVL3' primer (SEQ ID NO: 61) as external primers, the PCR reaction mixture was covered with 50 μ l of mineral oil. After initially denaturing for 3 minutes at 94°C, the reaction solution was incubated for 30 cycles of 1 minute at 94°C, 1 minute at 55°C and 1 minute at 72°C, followed finally by incubation for 10 minutes at 72°C (see Fig. 3).

15 A DNA fragment containing approximately 400 base pairs was purified using 1.5% low melting point agarose gel, digested with HindIII and BamHI and cloned into HEF expression vector HEF-VL-gk (Fig. 1). After determining the DNA sequence using EF-1 primer (SEQ ID NO: 66) and KIP primer (SEQ ID NO: 68), the plasmid that contained a DNA fragment that codes for the correct amino acid sequence of the L chain V region was named HEF-RVLa-gk. The amino acid sequence and nucleotide sequence of the L chain V region contained in this plasmid HEF-RVLa-gk are shown in SEQ ID NO: 62.

20 Version "b" (RVLb) was amplified by PCR using mutagen primers FTY1 (SEQ ID NO: 63) and FTY2 (SEQ ID NO: 64), designed so that phenylalanine at position 71 was replaced by tyrosine, RVL5' (SEQ ID NO: 60) and RVL3' (SEQ ID NO: 61) for the primers that define both ends, and plasmid HEF-RVLa-gk as the template DNA to obtain plasmid HEF-RVlb-gk. The amino acid sequence and nucleotide sequence of the L chain V region contained in this plasmid HEF-RVlb-gk are shown in SEQ ID NO: 65.

25 In order to evaluate the antigen binding activity of each chain of the reshaped human WS-4 antibody, COS cells were first simultaneously transfected in the manner previously described in relation to expression vector HEF-RVLa-gk for version "a" of the L chain of reshaped human WS-4 antibody, and expression vector HEF-chWS4H-gy1 for the H chain of chimeric WS-4 antibody. After collecting the culture medium as previously described, the amount of antibody produced and antigen binding activity were measured for the antibodies produced using the method described in the section on ELISA in the above Example 4. Those results are shown in Fig. 4. As shown in Fig. 4, it was confirmed that there was no difference in antigen binding activity between chimeric antibody (chL/chH), used as the positive control, and antibody consisting of a reshaped L chain and chimeric H chain (RVLa/chH).

30 At the same time, in order to evaluate the combination of expression vector HEF-chWS4L-gk for the L chain of chimeric WS-4 antibody and version "a" of the H chain of reshaped human WS-4 antibody, both were simultaneously CO-transfected into COS cells and the amount of antibody produced and antigen binding activity were measured for the resulting antibody using the method described in the section on "ELISA" in the above Example 4. Antigen binding activity was not demonstrated for this antibody (chL/RVHa) (see Fig. 4).

35 As previously described, since version "a" of the L chain of reshaped human WS-4 antibody exhibited antigen binding activity equal to that of the L chain of chimeric WS-4 antibody, evaluation of each version of all reshaped H chains was performed by simultaneously transfected COS cells with each version of the reshaped H chain and version "a" of the L chain of reshaped human WS-4 antibody (RVLa).

40 The result was that those antibodies having versions "b", "d", "e", "f", "g" and "h" of the reshaped H chain exhibited antigen binding activity comparable to that of chimeric WS-4 antibody (chL/chH) used as the positive control, thus indicating that this combination forms a functional antigen binding site in human antibody. However, with respect to the amount of antibody produced, all versions were produced in lesser amount than chimeric WS-4 antibody (chL/chH) with the exception of version "g" (RVHg). Furthermore, antigen binding activity was not observed in antibody having H chain version "c" (see Fig. 5).

45 Based on these findings, it was concluded that antibody having version "a" of the L chain of reshaped human WS-4 antibody (RVLa) and version "g" of the H chain of reshaped human WS-4 antibody reforms a functional antigen binding site that exhibits favorable antigen binding activity, and that the amount of antibody produced is comparable to chimeric WS-4 antibody (chL/chH) following simultaneous transfection into COS cells.

50 Next, an evaluation of version "b" of the L chain of reshaped human WS-4 antibody (RVLb) was performed by simultaneously transfecting COS cells with each version of the H chain with version "b" of the L chain of reshaped human WS-4 antibody (RVLb). The result showed that only antibody having version "g" of the H chain of reshaped human WS-4 antibody (RVLb/RVHg) exhibited antigen binding activity comparable to chimeric WS-4 antibody (chL/chH) used as the positive control, and it was concluded that this combination forms a functional antigen binding site in human anti-

body. In addition, with respect to amount of antibody produced, all versions were produced in lesser amount than chimeric WS-4 antibody (chL/chH) with the exception of version "g" (RVHg) (see Fig. 6).

5 In the above-mentioned evaluation, the two types of reshaped human antibody (RVLa/RVHg and RVLb/RVHg) that exhibited binding activity to human IL-8 and extent of production comparable to that of chimeric WS-4 antibody (chL/chH) were respectively purified with a Protein A column, after which binding activity was evaluated accurately using the method described in the section on ELISA in Example 4. The result showed that chimeric WS-4 antibody (chL/chH), RVLa/RVHg antibody and RVLb/RVHg antibody all exhibited the same extents of binding activity (see Fig. 7).

10 Based on these findings, it was concluded that antibody having either version "a" (RVLa) or version "b" (RVLb) of the L chain of reshaped human WS-4 antibody and version "g" (RVHg) of the H chain of reshaped human WS-4 antibody reforms a functional antigen binding site that a level of exhibits favorable antigen binding activity, and that a level of antibody production comparable to that of chimeric WS-4 antibody (chL/chH) was exhibited following simultaneous transfection into COS cells.

15 The inhibitory activity on IL-8 binding to IL-8 receptors of reshaped human antibody consisting of version "a" (RVLa) of the H chain and version "g" (RVHg) of the H chain of reshaped human WS-4 antibody, or version "b" (RVLb) of said L chain and version "g" (RVHg) of said H chain, was evaluated by ligand receptor binding inhibition assay.

20 Approximately 100 ml of heparinized blood sample from normal subjects was layered in 35 ml aliquots onto 15 ml of Mono-Poly separation solution (ICN Biomedicals), and the human neutrophil layer was isolated by centrifugation according to the instructions provided. After washing these cells with RPMI-1640 medium containing 1% BSA, contaminating erythrocytes were removed with 150 mM ammonium chloride solution. After centrifuging, the cells were washed with RPMI-1640 medium containing 1% BSA and resuspended at a concentration of 2×10^7 cells/ml. The neutrophil content of this cell suspension was found to be 95% or more as a result of measuring after staining smear specimens prepared using Cytospin (Shandon) with Diff-Quik stain (Green Cross).

25 The above-mentioned neutrophil suspension was centrifuged and resuspended at a concentration of 2×10^7 cells/ml with binding buffer (D-PBS containing 1% BSA and 0.1% sodium azide). At this time, SK2 chimeric antibody having an Fc portion identical to that of the human antibody of the present invention (see International Patent Application No. PCT/JP94/00859) and its antigen, human IL-6, were added to concentrations of approximately 50 μ g/ml and approximately 40 ng/ml, respectively, and incubated for 30 minutes in an ice bath for the purpose of pre-saturating the Fc receptors on the neutrophils.

30 IL-8 radioactively labeled with ^{125}I (74 TBq/mmol, Amersham) and non-labeled IL-8 (Amersham) prepared by mixing in binding buffer at concentrations of 4 ng/ml each. Chimeric WS-4 antibody (chL/chH), reshaped human antibody (RVLa/RVHg and RVLb/RVHg), negative control human antibody (PAESEL + LOREI) or positive control mouse WS-4 antibody was respectively diluted with binding buffer at concentrations between 2000 ng/ml and approximately 8 ng/ml in stepwise, 2-fold dilutions. 50 μ l of IL-8 solution and 50 μ l of each of the antibody solutions were incubated for 30 minutes in an ice bath. Next, 100 μ l of the above-mentioned neutrophil suspension was added and incubation was continued further for 1 hour with mixing every 15 minutes. Following incubation, the cell suspension was layered onto 200 μ l of 20% sucrose solution followed by centrifugation and freezing. In order to measure the IL-8 bound to the cells, the cell sediment was cut away and radioactivity was measured with a gamma counter (Aroka). Those results are shown in Fig. 8.

35 40 Antibody having version "a" of the L chain (RVLa) and version "g" of the H chain (RVHg) of reshaped human WS-4 antibody, or version "b" of said L chain and version "g" of said H chain, was clearly shown to have binding inhibitory activity comparable to that of chimeric antibody (chL/chH) in respect of the binding of IL-8 to IL-8 receptors.

45 Furthermore, the *Escherichia coli* having the above-mentioned plasmid HEF-RVLa-g κ was deposited as *Escherichia coli* DH5 α (HEF-RVLa-g κ), and the *Escherichia coli* containing plasmid HEF-RVHg-g γ 1 was deposited as *Escherichia coli* JM109 (HEF-RVHg-g γ 1) at the Bioengineering Industrial Technology Research Institute of the Agency of Industrial Science and Technology (1-1-3 Higashi, Tsukuba, Ibaraki, Japan) on July 12, 1994 under the respective names FERM BP-4738 and FERM BP-4741 based on the provisions of the Budapest Convention.

Reference Example 1: Preparation of Hybridoma WS-4

50 Hybridoma that produces anti-human IL-8 monoclonal antibody was prepared by fusing spleen cells of BALB/c mice immunized with human IL-8 and mouse myeloma cells P3x63-Ag8.653 according to routine methods using polyethylene glycol. Screening was performed using the activity of binding with human IL-8 as the criterion to establish the hybridoma WS-4 (Ko, Y.C. et al., *J. Immunol. Methods*, 149, 227-235, 1992).

55 INDUSTRIAL APPLICABILITY

The present invention provides reshaped human antibody against human IL-8, and in this antibody, the CDR of the V region of human antibody is substituted with the CDR of mouse monoclonal antibody against human IL-8. Since the

majority of this reshaped human antibody is of human origin and CDR inherently having low antigenicity, the reshaped human antibody of the Present invention has low antigenicity to humans, and for this reason can be expected to be useful in medical treatment.

5 List of Microorganisms Deposited under the Provisions of Article 13 bis of the Patent Cooperation Treaty International Deposit Authority:

Name: National Institute of Bioscience and Human-Technology Agency of Industrial Science and Technology
Address: 1-3 Higashi 1-chome, Tsukuba, Ibaraki, Japan

10 Deposit Numbers and Deposition Dates:

(1) Escherichia coli DH5 α (HEF-RVLa-g κ)

Deposit no.: FERM BP-4738

15 Deposition date: July 12, 1994

(2) Escherichia coli DH5 α (HEF-chWS4L-g κ)

Deposit no.: FERM BP-4739

Deposition date: July 12, 1994

(3) Escherichia coli JM109 (HEF-chWS4H-g γ 1)

20 Deposit no.: FERM BP-4740

Deposition date: July 12, 1994

(4) Escherichia coli JM109 (HEF-RVHg-g γ 1)

Deposit no.: FERM BP-4741

Deposition date: July 12, 1994

25

30

35

40

45

50

55

SEQUENCE LISTING

5
SEQ ID NO: 1
SEQUENCE LENGTH: 40
SEQUENCE TYPE: Nucleic acid
10 STRANDEDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
15 NAME OF SEQUENCE: MKV1
SEQUENCE
ACTAGTCGAC ATGAAAGTTGC CTGTTAGGCT GTTGGTGCTG 40
20 SEQ ID NO: 2
SEQUENCE LENGTH: 39
SEQUENCE TYPE: Nucleic acid
25 STRANDEDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
30 NAME OF SEQUENCE: MKV2
SEQUENCE
ACTAGTCGAC ATGGAGWCAG ACACACTCCT GYTATGGGT 39
35 SEQ ID NO: 3
SEQUENCE LENGTH: 40
SEQUENCE TYPE: Nucleic acid
40 STRANDEDNESS: Single
45 TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: MKV3
50 SEQUENCE
ACTAGTCGAC ATGAGTGTGC TCACTCAGGT CCTGGSGTTG 40
55

SEQ ID NO: 4
SEQUENCE LENGTH: 43
5 SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single
10 TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA.
NAME OF SEQUENCE: MKV4
15 SEQUENCE
ACTAGTCGAC ATGAGGRCCC CTGCTCAGWT TYTTGGMWTC TTG 43
SEQ ID NO: 5
20 SEQUENCE LENGTH: 40
SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single
25 TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: MKV5
30 SEQUENCE
ACTAGTCGAC ATGGATTTC AGGTGCAGAT TWTCAAGCTTC 40
35 SEQ ID NO: 6
SEQUENCE LENGTH: 37
SEQUENCE TYPE: Nucleic acid
40 STRANDEDNESS: Single
TOPOLOGY: Linear
45 MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: MKV6
SEQUENCE
50 ACTAGTCGAC ATGAGGTCY YTGYTSAGYT YCTGRGG 37
SEQ ID NO: 7
55

SEQUENCE LENGTH: 41

SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: Single

TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: MKV7

SEQUENCE

15 ACTAGTCGAC ATGGGCWTCA AGATGGAGTC ACAKWYYCWG G 41

SEQ ID NO: 8

SEQUENCE LENGTH: 41

20 SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: Single

TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: MKV8

SEQUENCE

30 ACTAGTCGAC ATGTGGGGAY CTKTTTYCMM TTTTCAATT G 41

SEQ ID NO: 9

35 SEQUENCE LENGTH: 35

SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: Single

40 TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

45 NAME OF SEQUENCE: MKV9

SEQUENCE

50 ACTAGTCGAC ATGGTRTCCW CASCTCAGTT CCTTG 35

SEQ ID NO: 10

SEQUENCE LENGTH: 37

55

SEQUENCE TYPE: Nucleic acid
5 STRANDEDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
10 NAME OF SEQUENCE: MKV10
SEQUENCE
ACTAGTCGAC ATGTATATAT GTTTGTTGTC TATTCT 37
15 SEQ ID NO: 11
SEQUENCE LENGTH: 38
SEQUENCE TYPE: Nucleic acid
20 STRANDEDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
25 NAME OF SEQUENCE: MKV11
SEQUENCE
ACTAGTCGAC ATGGAAGCCC CAGCTCAGCT TCTCTTCC 38
30 SEQ ID NO: 12
SEQUENCE LENGTH: 27
35 SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single
40 TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: MKC
45 SEQUENCE
GGATCCCGGG TGGATGGTGG GAAGATG 27
SEQ ID NO: 13
50 SEQUENCE LENGTH: 37
SEQUENCE TYPE: Nucleic acid
55

STRANDEDNESS: Single
5
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: MHV1
10
SEQUENCE
ACTAGTCGAC ATGAAATGCA GCTGGGTCA STTCTTC 37
SEQ ID NO: 14
15
SEQUENCE LENGTH: 36
SEQUENCE TYPE: Nucleic acid
20
STRANDEDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
25
NAME OF SEQUENCE: MHV2
SEQUENCE
ACTAGTCGAC ATGGGATGGA GCTRTATCAT SYTCTT 36
30
SEQ ID NO: 15
SEQUENCE LENGTH: 37
SEQUENCE TYPE: Nucleic acid
35
STRANDEDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
40
NAME OF SEQUENCE: MHV3
SEQUENCE
ACTAGTCGAC ATGAAGWTGT GGTTAAACTG GGTTTTT 37
45
SEQ ID NO: 16
SEQUENCE LENGTH: 35
50
SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single

55

TOPOLOGY: Linear
5 MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: MHV4
SEQUENCE
10 ACTAGTCGAC ATGRACATTG GGYTCAGCTT GRTTT 35
SEQ ID NO: 17
SEQUENCE LENGTH: 40
15 SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single
TOPOLOGY: Linear
20 MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: MHV5
SEQUENCE
25 ACTAGTCGAC ATGGACTCCA GGCTCAATT AGTTTCCTT 40
SEQ ID NO: 18
SEQUENCE LENGTH: 37
30 SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single
TOPOLOGY: Linear
35 MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: MHV6
SEQUENCE
40 ACTAGTCGAC ATGGCTGTCY TRGSGCTRCT CTTCTGC 37
SEQ ID NO: 19
45 SEQUENCE LENGTH: 36
SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single
50 TOPOLOGY: Linear

55

5 MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: MHV7
SEQUENCE
ACTAGTCGAC ATGGRATGGA GCKGGRTCTT TMTCTT 36
10 SEQ ID NO: 20
SEQUENCE LENGTH: 33
SEQUENCE TYPE: Nucleic acid
15 STRANDEDNESS: Single
TOPOLOGY: Linear
20 MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: MHV8
SEQUENCE
ACTAGTCGAC ATGAGAGTGC TGATTCTTT GTG 33
25 SEQ ID NO: 21
SEQUENCE LENGTH: 40
SEQUENCE TYPE: Nucleic acid
30 STRANDEDNESS: Single
TOPOLOGY: Linear
35 MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: MHV9
SEQUENCE
ACTAGTCGAC ATGGMTTGGG TGTGGAMCTT GCTATTCTG 40
40 SEQ ID NO: 22
SEQUENCE LENGTH: 37
SEQUENCE TYPE: Nucleic acid
45 STRANDEDNESS: Single
50 TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
55

NAME OF SEQUENCE: MHV10

SEQUENCE

5 ACTAGTCGAC ATGGGCAGAC TTACATTCTC ATTCCCTG 37

SEQ ID NO: 23

10 SEQUENCE LENGTH: 38

SEQUENCE TYPE: Nucleic acid

15 STRANDEDNESS: Single

TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: MHV11

20 SEQUENCE

ACTAGTCGAC ATGGATTTG GGCTGATTT TTTTATTG 38

SEQ ID NO: 24

25 SEQUENCE LENGTH: 37

SEQUENCE TYPE: Nucleic acid

30 STRANDEDNESS: Single

TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

35 NAME OF SEQUENCE: MHV12

SEQUENCE

40 ACTAGTCGAC ATGATGGTGT TAAGTCTTCT GTACCTG 37

SEQ ID NO: 25

SEQUENCE LENGTH: 28

45 SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: Single

TOPOLOGY: Linear

50 MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: MHC

55

SEQUENCE

CAG CTC CTG GTC TAT AAT GCA AAA ACC TTA GCA GAT GGT GTG TCA TCA 240
 Gln Leu Leu Val Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Ser Ser
 5 45 50 55 60
 AGG TTC AGT GGC AGT GGA TCA GGC ACA CAG TTT TCT CTG CGG ATC AGC 288
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Phe Ser Leu Arg Ile Ser
 10 65 70 75
 AGC CTG CAG CCT GAA GAT TTT GGG AGT TAT TAC TGT CAA CAT CAT TTT 336
 Ser Leu Gln Pro Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His His Phe
 15 80 85 90
 GGT TTT CCT CGG ACG TTC GGT GGA GGC ACC AAG CTG GAA CTC AAA C 382
 Gly Phe Pro Arg Thr Phe Gly Gly Thr Lys Leu Glu Leu Lys
 20 95 100 105
 SEQ ID NO: 27
 SEQUENCE LENGTH: 424
 25 SEQUENCE TYPE: Nucleic acid
 STRANDEDNESS: Double
 30 TOPOLOGY: Linear
 MOLECULE TYPE: cDNA
 NAME OF SEQUENCE: WS4VH
 35 Source
 Organism: Mouse
 Immediate source
 40 Clone: pUC-WS4-VH
 Characteristics: 1..57 sig peptide
 45 58..424 mat peptide
 Sequence
 ATG AAG TTG TGG TTA AAC TGG GTT TTT CTT GTG ACA CTT TTA AAT GGT 48
 50 Met Lys Leu Trp Leu Asn Trp Val Phe Leu Val Thr Leu Leu Asn Gly
 -19 -15 -10 -5
 55

ATC CAG TGT GAG GTG AAA CTG GTG GAG TCT GGA GGA GGC TTG ATA CAG 96
 Ile Gln Cys Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Ile Gln
 5 -1 1 5 10
 CCT GGG GAT TCT CTG AGA CTC TCC TGT GTA ACC TCT GGG TTC ACC TTC 144
 Pro Gly Asp Ser Leu Arg Leu Ser Cys Val Thr Ser Gly Phe Thr Phe
 10 15 20 25
 AGT GAT TAC TAC CTG AGC TGG GTC CGC CAG CCT CCA GGA AAG GCA CTT 192
 Ser Asp Tyr Tyr Leu Ser Trp Val Arg Gln Pro Pro Gly Lys Ala Leu
 15 30 35 40 45
 GAG TGG GTG GGT CTC ATT AGA AAC AAA GCC AAT GGT TAC ACA AGA GAG 240
 Glu Trp Val Gly Leu Ile Arg Asn Lys Ala Asn Gly Tyr Thr Arg Glu
 20 50 55 60
 TAC AGT GCA TCT GTG AAG GGT CGG TTC ACC ATC TCC AGA GAT GAT TCC 288
 Tyr Ser Ala Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser
 25 65 70 75
 CAA AGC ATC CTC TAT CTT CAA ATG AAC ACC CTG AGA GGT GAG GAC AGT 336
 Gln Ser Ile Leu Tyr Leu Gln Met Asn Thr Leu Arg Gly Glu Asp Ser
 30 80 85 90
 GCC ACT TAT TAC TGT GCA CGA GAG AAC TAT AGG TAC GAC GTA GAG CTT 384
 Ala Thr Tyr Tyr Cys Ala Arg Glu Asn Tyr Arg Tyr Asp Val Glu Leu
 35 95 100 105
 GCT TAC TGG GGC CAA GGG ACT CTG GTC ACT GTC TCT GCA G 424
 Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
 40 110 115 120
 SEQ ID NO: 28
 SEQUENCE LENGTH: 34
 45 SEQUENCE TYPE: Nucleic acid
 STRANDEDNESS: Single
 TOPOLOGY: Linear
 50 MOLECULE TYPE: Synthetic DNA
 NAME OF SEQUENCE: chVL backward primer

55

SEQUENCE
5 ACAAAGCTTC CACCATGAGT GTGCTCACTC AGGT 34
SEQ ID NO: 29
SEQUENCE LENGTH: 37
10 SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single
TOPOLOGY: Linear
15 MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: chVH backward primer
SEQUENCE
20 GATAAGCTTC CACCATGAAG TTGTGGTTAA ACTGGGT 37
SEQ ID NO: 30
SEQUENCE LENGTH: 37
25 SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single
TOPOLOGY: Linear
30 MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: chVL forward primer
SEQUENCE
35 CTTGGATCCA CTCACGTTTG AGTTCCAGCT TGGTGCC 37
SEQ ID NO: 31
40 SEQUENCE LENGTH: 37
SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single
45 TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: chVH forward primer
SEQUENCE
50

55

5 GTCGGATCCA CTCACCTGCA GAGACAGTGA CCAGAGT 37
SEQ ID NO: 32
SEQUENCE LENGTH: 137
SEQUENCE TYPE: Nucleic acid
10 STRANDEDNESS: Single
TOPOLOGY: Linear
15 MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: HF1
SEQUENCE
TAAGCTTCCA CCATGGAGTT TGGGCTGAGC TGGGTTTCC TTGTTGCTAT TTTAAAGGGT 60
20 GTCCAGTGTG AAGTGCAGCT GTTGGAGTCT GGGGGAGGCT TGGTCCAGCC TGGGGGTTCT 120
CTGAGACTCT CATGTGC 137
SEQ ID NO: 33
SEQUENCE LENGTH: 143
SEQUENCE TYPE: Nucleic acid
30 STRANDEDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
35 NAME OF SEQUENCE: HF2
SEQUENCE
GCACTGTACT CTCTTGTGTA ACCATTGGCT TTGTTCTAA TGAGACCCAC CAACTCTAGC 60
40 CCTTCCCTT GAGCTTGGCG GACCCAGCTC AGGTAGTAAT CACTGAAGGT GAATCCAGAG 120
GCAGCACATG AGAGTCTAG AGA 143
SEQ ID NO: 34
SEQUENCE LENGTH: 113
SEQUENCE TYPE: Nucleic acid
45 STRANDEDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: HF3

SEQUENCE

5 TACACAAGAG ACTACAGTGC ATCTGTGAAG CCCAGACTTA CCATCTCAAG AGAAGATTCA 60
AAGAACACCC TGTATCTGCA AATGAGCAGC CTGAAAACCG AAGACTTGGC CGT 113

10 SEQ ID NO: 35

15 SEQUENCE LENGTH: 117

SEQUENCE TYPE: Nucleic acid

20 STRANDEDNESS: Single

TOPOLOGY: Linear

25 MOLECULE TYPE: Synthetic DNA

30 NAME OF SEQUENCE: HF4

SEQUENCE

35 TCGGATCCAC TCACCTGAGG AGACGGTGAC CAGGGTTCCC TGGCCCCAGT AAGCAAGCTC 60
TACGTCGTAG CGATAGTTCT CTCTAGCACA GTAATACACG GCCAAGTCTT CGGTTT 117

40 SEQ ID NO: 36

50 SEQUENCE LENGTH: 37

SEQUENCE TYPE: Nucleic acid

55 STRANDEDNESS: Single

TOPOLOGY: Linear

60 MOLECULE TYPE: Synthetic DNA

45 NAME OF SEQUENCE: RVH5' primer

SEQUENCE

65 GATAAGCTTC CACCATGGAG TTTGGGCTGA GCTGGT 37

70 SEQ ID NO: 37

SEQUENCE LENGTH: 31

75 SEQUENCE TYPE: Nucleic acid

80 STRANDEDNESS: Single

TOPOLOGY: Linear

85

5 MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: RVH3' primer

SEQUENCE

10 GTCGGATCCA CTCACCTGAG GAGACGGTGA C

31

SEQ ID NO: 38

15 SEQUENCE LENGTH: 424

SEQUENCE TYPE: Nucleic acid

20 STRANDEDNESS: Double

TOPOLOGY: Linear

25 MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: RVHa

Source

30 Organism: Mouse and human

Immediate source

35 Clone: HEF-RVHa-gyl

40 Amino acid -19--1:leader

Amino acid 1-30:FR1

45 Amino acid 31-35:CDR1

Amino acid 36-49:FR2

Amino acid 50-68:CDR2

50 Amino acid 69-100:FR3

Amino acid 101-111:CDR3

55 Amino acid 112-122:FR4

Sequence

ATG GAG TTT GGG CTG AGC TGG GTT TTC CTT GTT GCT ATT TTA AAG GGT

48

50 Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Lys Gly

-19 -15 -10 -5

55

	GTC CAG TGT GAA GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTC CAG	96
	Val Gln Cys Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln	
5	-1 1 5 10	
	CCT GGG GGT TCT CTG AGA CTC TCA TGT GCT GCC TCT GGA TTC ACC TTC	144
	Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe	
10	15 20 25	
	AGT GAT TAC TAC CTG AGC TGG GTC CGC CAA GCT CAA GGG AAA GGG CTA	192
	Ser Asp Tyr Tyr Leu Ser Trp Val Arg Gln Ala Gln Gly Lys Gly Leu	
15	30 35 40 45	
	GAG TTG GTG GGT CTC ATT AGA AAC AAA GCC AAT GGT TAC ACA AGA GAG	240
	Glu Leu Val Gly Leu Ile Arg Asn Lys Ala Asn Gly Tyr Thr Arg Glu	
20	50 55 60	
	TAC AGT GCA TCT GTG AAG GGC AGA CTT ACC ATC TCA AGA GAA GAT TCA	288
	Tyr Ser Ala Ser Val Lys Gly Arg Leu Thr Ile Ser Arg Glu Asp Ser	
25	65 70 75	
	AAG AAC ACG CTG TAT CTG CAA ATG AGC AGC CTG AAA ACC GAA GAC TTG	336
	Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu	
30	80 85 90	
	GCC GTG TAT TAC TGT GCT AGA GAG AAC TAT CGC TAC GAC GTA GAG CTT	384
	Ala Val Tyr Tyr Cys Ala Arg Glu Asn Tyr Arg Tyr Asp Val Glu Leu	
35	95 100 105	
	GCT TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA G	424
	Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser	
40	110 115 120	
	SEQ ID NO: 39	
	SEQUENCE LENGTH: 34	
45	SEQUENCE TYPE: Nucleic acid	
	STRANDEDNESS: Single	
	TOPOLOGY: Linear	
50	MOLECULE TYPE: Synthetic DNA	
	NAME OF SEQUENCE: LTW1	

55

SEQUENCE

GGCTAGAGTG GGTGGGTCTC ATTAGAAACA AAGC

34

5 SEQ ID NO: 40

SEQUENCE LENGTH: 36

10 SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: Single

TOPOLOGY: Linear

15 MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: LTW2

SEQUENCE

20 GAGACCCACC CACTCTAGCC CTTTCCCTTG AGCTTG

36

SEQ ID NO: 41

SEQUENCE LENGTH: 424

25 SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: Double

30 TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: RVHb

35 Source

Organism: Mouse and human

40 Immediate source

Clone: HEF-RVHb-gy1

Amino acid -19--1:leader

45 Amino acid 1-30:FR1

Amino acid 31-35:CDR1

Amino acid 36-49:FR2

50 Amino acid 50-68:CDR2

Amino acid 69-100:FR3

55

Amino acid 101-111:CDR3

Amino acid 112-122:FR4

5

Sequence

ATG GAG TTT GGG CTG AGC TGG GTT TTC CTT GTT GCT ATT TTA AAG GGT	48
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Lys Gly	
10 -19 -15 -10 -5	
GTC CAG TGT GAA GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTC CAG	96
Val Gln Cys Glu Val Gln Leu Leu Glu Ser Gly Gly Leu Val Gln	
15 -1 1 5 10	
CCT GGG GGT TCT CTG AGA CTC TCA TGT GCT GCC TCT GGA TTC ACC TTC	144
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe	
20 15 20 25	
AGT GAT TAC TAC CTG AGC TGG GTC CGC CAA GCT CAA GGG AAA GGG CTA	192
Ser Asp Tyr Tyr Leu Ser Trp Val Arg Gln Ala Gln Gly Lys Gly Leu	
25 30 35 40 45	
GAG TGG GTG GGT CTC ATT AGA AAC AAA GCC AAT GGT TAC ACA AGA GAG	240
Glu Trp Val Gly Leu Ile Arg Asn Lys Ala Asn Gly Tyr Thr Arg Glu	
30 50 55 60	
TAC AGT GCA TCT GTG AAG GGC AGA CTT ACC ATC TCA AGA GAA GAT TCA	288
Tyr Ser Ala Ser Val Lys Gly Arg Leu Thr Ile Ser Arg Glu Asp Ser	
35 65 70 75	
AAG AAC ACG CTG TAT CTG CAA ATG AGC AGC CTG AAA ACC GAA GAC TTG	336
Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu	
40 80 85 90	
GCC GTG TAT TAC TGT GCT AGA GAG AAC TAT CGC TAC GAC GTA GAG CTT	384
Ala Val Tyr Tyr Cys Ala Arg Glu Asn Tyr Arg Tyr Asp Val Glu Leu	
45 95 100 105	
GCT TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA G	424
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser	
50 110 115 120	
SEQ ID NO: 42	
SEQUENCE LENGTH: 32	

55

SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single
5 TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
10 NAME OF SEQUENCE: QTP1
SEQUENCE
TGGGTCCGCC AAGCTCCAGG GAAAGGGCTA GA 32
15 SEQ ID NO: 43
SEQUENCE LENGTH: 32
SEQUENCE TYPE: Nucleic acid
20 STRANDEDNESS: Single
TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
25 NAME OF SEQUENCE: QTP2
SEQUENCE
TCTAGCCCTT TCCCTGGAGC TTGGCCGACC CA 32
30 SEQ ID NO: 44
SEQUENCE LENGTH: 424
SEQUENCE TYPE: Nucleic acid
35 STRANDEDNESS: Double
TOPOLOGY: Linear
40 MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: RVHc
45 Source
Organism: Mouse and human
Immediate source
Clone: HEF-RVHc-gyl
Amino acid -19--1:leader

55

5 Amino acid 1-30:FR1
 10 Amino acid 31-35:CDR1
 15 Amino acid 36-49:FR2
 20 Amino acid 50-68:CDR2
 25 Amino acid 69-100:FR3
 30 Amino acid 101-111:CDR3
 35 Amino acid 112-122:FR4

15 Sequence

40 ATG GAG TTT GGG CTG AGC TGG GTT TTC CTT GTT GCT ATT TTA AAG GGT 48
 45 Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Lys Gly
 50 -19 -15 -10 -5
 55 GTC CAG TGT GAA GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTC CAG 96
 60 Val Gln Cys Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln
 65 -1 5 10
 70 CCT GGG GGT TCT CTG AGA CTC TCA TGT GCT GCC TCT GGA TTC ACC ACC 144
 75 Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
 80 15 20 25
 85 AGT GAT TAC TAC CTG AGC TGG GTC CGC CAA GCT CCA GGG AAA GGG CTA 192
 90 Ser Asp Tyr Tyr Leu Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 95 30 35 40 45
 100 GAG TTG GTG GGT CTC ATT AGA AAC AAA GCC AAT GGT TAC ACA AGA GAG 240
 105 Glu Leu Val Gly Leu Ile Arg Asn Lys Ala Asn Gly Tyr Thr Arg Glu
 110 50 55 60
 115 TAC AGT GCA TCT GTG AAG GCC AGA CTT ACC ATC TCA AGA GAA GAT TCA 288
 120 Tyr Ser Ala Ser Val Lys Gly Arg Leu Thr Ile Ser Arg Glu Asp Ser
 125 65 70 75
 130 AAG AAC ACG CTG TAT CTG CAA ATG AGC AGC CTG AAA ACC GAA GAC TTG 336
 135 Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu
 140 80 85 90

5 GCC GTG TAT TAC TGT GCT AGA GAG AAC TAT CGC TAC GAC GTC GAG CTT 384
 Ala Val Tyr Tyr Cys Ala Arg Glu Asn Tyr Arg Tyr Asp Val Glu Leu

95 95 100 105

10 GCT TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA G 424
 Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

110 110 115 120

15 SEQ ID NO: 45

SEQUENCE LENGTH: 424

15 SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: Double

20 TOPOLOGY: Linear

20 MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: RVHd

25 Source

Organism: Mouse and human

Immediate source

30 Clone: HEF-RVHd-gy1

Amino acid -19--1:leader

35 Amino acid 1-30:FR1

Amino acid 31-35:CDR1

Amino acid 36-49:FR2

40 Amino acid 50-68:CDR2

Amino acid 69-100:FR3

Amino acid 101-111:CDR3

45 Amino acid 112-122:FR4

Sequence

50 ATG GAG TTT GGG CTG AGC TGG GTT TTC CTT GTT GCT ATT TTA AAG GGT 48

50 Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Lys Gly

-19 -15 -10 -5

55

EP 0 770 628 A1

GTC CAG TGT GAA GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTC CAG
 Val Gln Cys Glu Val Gln Leu Leu Glu Ser Gly Gly Leu Val Gln
 5 -1 1 5 10
 CCT GGG GGT TCT CTG AGA CTC TCA TGT GCT GCC TCT GGA TTC ACC TTC
 Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
 10 15 20 25
 AGT GAT TAC TAC CTG AGC TGG GTC CGC CAA GCT CCA GGG AAA GGG CTA
 Ser Asp Tyr Tyr Leu Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 15 30 35 40 45
 GAG TGG GTG GGT CTC ATT AGA AAC AAA GCC AAT GGT TAC ACA AGA GAG
 Glu Trp Val Gly Leu Ile Arg Asn Lys Ala Asn Gly Tyr Thr Arg Glu
 20 25 30 35 40 45
 TAC AGT GCA TCT GTG AAG GGC AGA CTT ACC ATC TCA AGA GAA GAT TCA
 Tyr Ser Ala Ser Val Lys Gly Arg Leu Thr Ile Ser Arg Glu Asp Ser
 25 30 35 40 45
 AAG AAC ACG CTG TAT CTG CAA ATG AGC AGC CTG AAA ACC GAA GAC TTG
 Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu
 30 35 40 45
 80 85 90 95 100 105
 GCC GTG TAT TAC TGT GCT AGA GAG AAC TAT CGC TAC GAC GTA GAG CTT
 Ala Val Tyr Tyr Cys Ala Arg Glu Asn Tyr Arg Tyr Asp Val Glu Leu
 35 40 45
 GCT TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA G
 Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 110 115 120
 40
 SEQ ID NO: 46
 SEQUENCE LENGTH: 26
 45
 SEQUENCE TYPE: Nucleic acid
 STRANDEDNESS: Single
 TOPOLOGY: Linear
 MOLECULE TYPE: Synthetic DNA
 NAME OF SEQUENCE: ATP1

55

SEQUENCE
5 TGGGTCCGCC AACCTCCAGG GAAAGG 26
SEQ ID NO: 47
SEQUENCE LENGTH: 26
10 SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single
TOPOLOGY: Linear
15 MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: ATP2
SEQUENCE
20 CCTTTCCCTG GAGGTTGGCG GACCCA 26
SEQ ID NO: 48
SEQUENCE LENGTH: 424
25 SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Double
30 TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: RVHe
35 Source
Organism: Mouse and human
40 Immediate source
Clone: HEF-RVHe-gy1
Amino acid -19--1:leader
45 Amino acid 1-30:FR1
Amino acid 31-35:CDR1
Amino acid 36-49:FR2
50 Amino acid 50-68:CDR2
Amino acid 69-100:FR3

55

Amino acid 101-111:CDR3

Amino acid 112-122:FR4

5

Sequence

ATG GAG TTT GGG CTG AGC TGG GTT TTC CTT GTT GCT ATT TTA AAG GGT	48
10 Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Lys Gly	
-19 -15 -10 -5	
15 GTC CAG TGT GAA GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTC CAG	96
Val Gln Cys Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln	
-1 1 5 10	
20 CCT GGG GGT TCT CTG AGA CTC TCA TGT GCT GCC TCT GGA TTC ACC TTC	144
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe	
15 20 25	
25 AGT GAT TAC TAC CTG AGC TGG GTC CGC CAA CCT CCA GGG AAA GGG CTA	192
Ser Asp Tyr Tyr Leu Ser Trp Val Arg Gln Pro Pro Gly Lys Gly Leu	
30 35 40 45	
30 GAG TGG GTG GGT CTC ATT AGA AAC AAA GCC AAT GGT TAC ACA AGA GAG	240
Glu Trp Val Gly Leu Ile Arg Asn Lys Ala Asn Gly Tyr Thr Arg Glu	
35 50 55 60	
35 TAC AGT GCA TCT GTG AAG GGC AGA CTT ACC ATC TCA AGA GAA GAT TCA	288
Tyr Ser Ala Ser Val Lys Gly Arg Leu Thr Ile Ser Arg Glu Asp Ser	
40 65 70 75	
40 AAG AAC ACG CTG TAT CTG CAA ATG AGC AGC CTG AAA ACC GAA GAC TTG	336
Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu	
45 80 85 90	
45 GCC GTG TAT TAC TGT GCT AGA GAG AAC TAT CGC TAC GAC GTA GAG CTT	384
Ala Val Tyr Tyr Cys Ala Arg Glu Asn Tyr Arg Tyr Asp Val Glu Leu	
50 95 100 105	
50 GCT TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA G	424
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser	
55 110 115 120	
55 SEQ ID NO: 49	
55 SEQUENCE LENGTH: 29	

55

SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single
5 TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
10 NAME OF SEQUENCE: GTA1
SEQUENCE
CAAGCTCCAG GGAAAGCGCT AGAGTGGGT 29
15 SEQ ID NO: 50
SEQUENCE LENGTH: 29
SEQUENCE TYPE: Nucleic acid
20 STRANDEDNESS: Single
TOPOLOGY: Linear
25 MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: GTA2
SEQUENCE
ACCCACTCTA GCGCTTCCCC TGGAGCTTG 29
30 SEQ ID NO: 51
SEQUENCE LENGTH: 424
35 SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Double
40 TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: RVHf
45 Source
Organism: Mouse and human
Immediate source
50 Clone: HEF-RVHf-gyl
Amino acid -19--1:leader

55

5	Amino acid	1-30:FR1
	Amino acid	31-35:CDR1
	Amino acid	36-49:FR2
10	Amino acid	50-68:CDR2
	Amino acid	69-100:FR3
	Amino acid	101-111:CDR3
15	Amino acid	112-122:FR4

Sequence

20	ATG GAG TTT GGG CTG AGC TGG GTT TTC CTT GTT GCT ATT TTA AAG GGT	48
	Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Lys Gly	
	-19 -15 -10 -5	
25	GTC CAG TGT GAA GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTC CAG	96
	Val Gln Cys Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln	
	-1 1 5 10	
30	CCT GGG GGT TCT CTG AGA CTC TCA TGT GCT GCC TCT GGA TTC ACC TTC	144
	Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe	
	15 20 25	
35	AGT GAT TAC TAC CTG AGC TGG GTC CGC CAA GCT CCA GGG AAA GCG CTA	192
	Ser Asp Tyr Tyr Leu Ser Trp Val Arg Gln Ala Pro Gly Lys Ala Leu	
	30 35 40 45	
40	GAG TGG GTG GGT CTC ATT AGA AAC AAA GCC AAT GGT TAC ACA AGA GAG	240
	Glu Trp Val Gly Leu Ile Arg Asn Lys Ala Asn Gly Tyr Thr Arg Glu	
	50 55 60	
45	TAC AGT GCÁ TCT GTG AAG GGC AGA CTT ACC ATC TCA AGA GAA GAT TCA	288
	Tyr Ser Ala Ser Val Lys Gly Arg Leu Thr Ile Ser Arg Glu Asp Ser	
	65 70 75	
50	AAG AAC ACG CTG TAT CTG CAA ATG AGC AGC CTG AAA ACC GAA GAC TTG	336
	Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu	
	80 85 90	

MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: RVHg

5
Source

Organism: Mouse and human

10
Immediate source

Clone: HEF-RVHg-gy1

Amino acid -19--1:leader

15
Amino acid 1-30:FR1

Amino acid 31-35:CDR1

20
Amino acid 36-49:FR2

Amino acid 50-68:CDR2

Amino acid 69-100:FR3

25
Amino acid 101-111:CDR3

Amino acid 112-122:FR4

Sequence

30
ATG GAG TTT GGG CTG AGC TGG GTT TTC CTT GTT GCT ATT TTA AAG GGT 48
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Lys Gly
-19 -15 -10 -535
GTC CAG TGT GAA GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTC CAG 96
Val Gln Cys Glu Val Gln Leu Leu Glu Ser Gly Gly Leu Val Gln
-1 1 5 1040
CCT GGG GGT TCT CTG AGA CTC TCA TGT GCT GCC TCT GGA TTC ACC TTC 144
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
15 20 2545
AGT GAT TAC TAC CTG AGC TGG GTC CGC CAA GCT CCA GGG AAA GGG CTA 192
Ser Asp Tyr Tyr Leu Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
30 35 40 4550
GAG TGG GTG GGT CTC ATT AGA AAC AAA GCC AAT GGT TAC ACA AGA GAG 240
Glu Trp Val Gly Leu Ile Arg Asn Lys Ala Asn Gly Tyr Thr Arg Glu
50 55 60

55

TAC AGT GCA TCT GTG AAG GGC AGA TTT ACC ATC TCA AGA GAA GAT TCA 288
 Tyr Ser Ala Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Glu Asp Ser
 5 65 70 75
 AAG AAC ACG CTG TAT CTG CAA ATG AGC AGC CTG AAA ACC GAA GAC TTG 336
 Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu
 10 80 85 90
 GCC GTG TAT TAC TGT GCT AGA GAG AAC TAT CGC TAC GAC GTC GAG CTT 384
 Ala Val Tyr Tyr Cys Ala Arg Glu Asn Tyr Arg Tyr Asp Val Glu Leu
 15 95 100 105
 GCT TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA G 424
 Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 20 110 115 120
 SEQ ID NO: 55
 SEQUENCE LENGTH: 424
 25 SEQUENCE TYPE: Nucleic acid
 STRANDEDNESS: Double
 30 TOPOLOGY: Linear
 MOLECULE TYPE: Synthetic DNA
 NAME OF SEQUENCE: RVHh
 35 Source
 Organism: Mouse and human
 Immediate source
 40 Clone: HEF-RVHh-gy1
 Amino acid -19--1:leader
 45 Amino acid 1-30:FR1
 Amino acid 31-35:CDR1
 Amino acid 36-49:FR2
 50 Amino acid 50-68:CDR2
 Amino acid 69-100:FR3

55

Amino acid 101-111:CDR3

Amino acid 112-122:FR4

5

Sequence

ATG GAG TTT GGG CTG AGC TGG GTT TTC CTT GTT GCT ATT TTA AAG GGT	48
Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Lys Gly	
10 -19 -15 -10 -5	
GTC CAG TGT GAA GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTC CAG	96
Val Gin Cys Glu Val Gln Leu Leu Glu Ser Gly Gly Leu Val Gln	
15 -1 1 5 10	
CCT GGG GGT TCT CTG AGA CTC TCA TGT GCT GCC TCT GGA TTC ACC TTC	144
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe	
20 15 20 25	
AGT GAT TAC TAC CTG AGC TGG GTC CGC CAA GCT CAA GGG AAA GGG CTA	192
Ser Asp Tyr Tyr Leu Ser Trp Val Arg Gln Ala Gln Gly Lys Gly Leu	
25 30 35 40 45	
GAG TGG GTG GGT CTC ATT AGA AAC AAA GCC AAT GGT TAC ACA AGA GAG	240
Glu Trp Val Gly Leu Ile Arg Asn Lys Ala Asn Gly Tyr Thr Arg Glu	
30 50 55 60	
TAC AGT GCA TCT GTG AAG GGC AGA TTT ACC ATC TCA AGA GAA GAT TCA	288
Tyr Ser Ala Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Glu Asp Ser	
35 65 70 75	
AAG AAC ACG CTG TAT CTG CAA ATG AGC AGC CTG AAA ACC GAA GAC TTG	336
Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Thr Glu Asp Leu	
40 80 85 90	
GCC GTG TAT TAC TGT GCT AGA GAG AAC TAT CGC TAC GAC GTA GAG CTT	384
Ala Val Tyr Tyr Cys Ala Arg Glu Asn Tyr Arg Tyr Asp Val Glu Leu	
45 95 100 105	
GCT TAC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC TCA G	424
Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser	
50 110 115 120	
SEQ ID NO: 56	
SEQUENCE LENGTH: 124	

55

SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: Single

5 TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

10 NAME OF SEQUENCE: LF1

SEQUENCE

TTGAAGCTTC CACCATGGGA TGGAGCTGTA TCATCCTCTT CTTGGTAGCA ACAGCTACAG 60

15 GTGTCCACTC CGACATCCAG ATGACCCAGA GCCCAAGCAG CCTGAGCGCC AGCGTAGGTG 120

ACAG 124

SEQ ID NO: 57

20 SEQUENCE LENGTH: 122

SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: Single

25 TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

30 NAME OF SEQUENCE: LF2

SEQUENCE

GCATTGTAGA TCAGCAGCTT TGGAGCCTTT CCTGGCTTCT GCTGGTACCA TGCTAAATAA 60

35 CTGTAATAAA TCTCGCTTGC TCGACAGGTG ATGGTCACTC TGTACCTAC GCTGGCGCTC 120

AG 122

SEQ ID NO: 58

40 SEQUENCE LENGTH: 121

SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: Single

45 TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

50 NAME OF SEQUENCE: LF3

SEQUENCE

55

AGCTGCTGAT CTACAATGCA AAAACCTTAG CAGATGGAGT GCCAAGCAGA TTCAGCGGTA 60
GCGGTAGCGG TACCGACTTC ACCTTCACCA TCAGCAGCCT CCAGCCAGAG GACATCGCTA 120
5 C 121

SEQ ID NO: 59

SEQUENCE LENGTH: 106

10 SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: Single

15 TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: LF4

20 SEQUENCE

GTAGGATCCA CTCACGTTG ATTCGACCT TGGTCCCTTG GCCGAACGTC CGAGGAAAAC 60
CAAAATGATG TTGGCAGTAG TAGGTACCGA TGTCCCTCTGG CTGGAG 106

25 SEQ ID NO: 60

SEQUENCE LENGTH: 20

SEQUENCE TYPE: Nucleic acid

30 STRANDEDNESS: Single

TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: RVLS'

35 SEQUENCE

40 TTGAAGCTTC CACCATGGGA 20

SEQ ID NO: 61

SEQUENCE LENGTH: 20

45 SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: Single

50 TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

55

NAME OF SEQUENCE: RVL'3

SEQUENCE

5 GTAGGATCCA CTCACGTTG

20

SEQ ID NO: 62

10 SEQUENCE LENGTH: 379

SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: Double

15 TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: RVLa

20 Source

Organism: Mouse and human

Immediate source

25 Clone: HEF-RVLa-gx

Amino acid -19--1:leader

30 Amino acid 1-23:FR1

Amino acid 24-34:CDR1

Amino acid 35-49:FR2

35 Amino acid 50-56:CDR2

Amino acid 57-88:FR3

Amino acid 89-97:CDR3

40 Amino acid 98-107:FR4

Sequence

45 ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT 48

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly

-19 -15 -10 -5

50 GTC CAC TCC GAC ATC CAG ATG ACC CAG AGC CCA AGC AGC CTG AGC GCC 96

Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala

-1 1 5 10

55

AGC GTA GGT GAC AGA GTG ACC ATC ACC TGT CGA GCA AGC GAG ATT ATT	144
Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Ile Ile	
5 15 20 25	
TAC AGT TAT TTA GCA TGG TAC CAG CAG AAG CCA GGA AAG GCT CCA AAG	192
Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys	
10 30 35 40 45	
CTG CTG ATC TAC AAT GCA AAA ACC TTA GCA GAT GGA GTG CCA AGC AGA	240
Leu Leu Ile Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg	
15 50 55 60	
TTC AGC GGT AGC GGT ACC GAC TTC ACC TTC ACC ATC AGC AGC	288
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser	
20 65 70 75	
CTC CAG CCA GAG GAC ATC GCT ACC TAC TAC TGC CAA CAT CAT TTT GGT	336
Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln His His Phe Gly	
25 80 85 90	
TTT CCT CGG ACG TTC GGC CAA GGG ACC AAG GTC GAA ATC AAA C	379
Phe Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys	
30 95 100 105	
SEQ ID NO: 63	
SEQUENCE LENGTH: 38	
35 SEQUENCE TYPE: Nucleic acid	
STRANDEDNESS: Single	
40 TOPOLOGY: Linear	
MOLECULE TYPE: Synthetic DNA	
NAME OF SEQUENCE: FTY1	
45 SEQUENCE	
AGCGGTAGCG GTACCGACTA CACCTTCACC ATCAGCAG	38
SEQ ID NO: 64	
SEQUENCE LENGTH: 38	
50 SEQUENCE TYPE: Nucleic acid	
STRANDEDNESS: Single	
55	

TOPOLOGY: Linear

MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: FTY2

SEQUENCE

10 CTGCTGATGG TGAAGGTGTA GTCGGTACCG CTACCGCT

38

SEQ ID NO: 65

SEQUENCE LENGTH: 379

15 SEQUENCE TYPE: Nucleic acid

STRANDEDNESS: Double

20 TOPOLOGY: Linear

20 MOLECULE TYPE: Synthetic DNA

NAME OF SEQUENCE: RVLb

25 Source

Organism: Mouse and human

Immediate source

30 Clone: HEF-RVLb-gx

Amino acid -19--1:leader

Amino acid 1-23:FR1

35 Amino acid 24-34:CDR1

Amino acid 35-49:FR2

40 Amino acid 50-56:CDR2

Amino acid 57-88:FR3

Amino acid 89-97:CDR3

45 Amino acid 98-107:FR4

Sequence

50 ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT

48

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly

-19 -15 -10 -5

55

	GTC CAC TCC GAC ATC CAG ATG ACC CAG AGC CCA AGC AGC CTG AGC GCC	96
	Val His Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala	
5	-1 1 5 10	
	AGC GTA GGT GAC AGA GTG ACC ATC ACC TGT CGA GCA AGC GAG ATT ATT	144
	Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Ile Ile	
10	15 20 25	
	TAC AGT TAT TTA GCA TCG TAC CAG CAG AAG CCA GGA AAG GCT CCA AAG	192
	Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys	
15	30 35 40 45	
	CTG CTG ATC TAC AAT GCA AAA ACC TTA GCA GAT GGA GTG CCA AGC AGA	240
	Leu Leu Ile Tyr Asn Ala Lys Thr Leu Ala Asp Gly Val Pro Ser Arg	
20	50 55 60	
	TTC AGC GGT AGC GGT AGC GGT ACC GAC TAC ACC TTC ACC ATC AGC AGC	288
	Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser	
25	65 70 75	
	CTC CAG CCA GAG GAC ATC GCT ACC TAC TAC TGC CAA CAT CAT TTT GGT	336
	Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln His His Phe Gly	
	80 85 90	
30	95 100 105	
35	SEQ ID NO: 66	
	SEQUENCE LENGTH: 18	
40	SEQUENCE TYPE: Nucleic acid	
	STRANDEDNESS: Single	
	TOPOLOGY: Linear	
45	MOLECULE TYPE: Synthetic DNA	
	NAME OF SEQUENCE: EFL	
	SEQUENCE	
50	CAGACAGTGG TTCAAAGT	18
	SEQ ID NO: 67	
55		

SEQUENCE LENGTH: 17
5 SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single
10 TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: HIP
15 SEQUENCE
GCCCCAAAGC CAAGGTC 17
SEQ ID NO: 68
20 SEQUENCE LENGTH: 20
SEQUENCE TYPE: Nucleic acid
STRANDEDNESS: Single
25 TOPOLOGY: Linear
MOLECULE TYPE: Synthetic DNA
NAME OF SEQUENCE: KIP
30 SEQUENCE
AACTCAATGC TTTAGGCAAA 20
35

40 **Claims**

1. A light chain (L chain) variable region (V region) of mouse monoclonal antibody against human Interleukin-8 (IL-8).
2. An L chain V region as set forth in claim 1 having the amino acid sequence or a portion thereof shown in SEQ ID NO: 26.
3. A heavy chain (H chain) V region of mouse monoclonal antibody against human IL-8.
4. An H chain V region as set forth in claim 3 having the amino acid sequence or a portion thereof shown in SEQ ID NO: 27.
5. Chimeric L chain comprising a human L chain constant region (C region), and an L chain V region of mouse monoclonal antibody against human IL-8.
- 55 6. Chimeric L chain as set forth in claim 5, wherein said mouse L chain V region has the amino acid sequence or a portion thereof shown in SEQ ID NO: 26.
7. Chimeric H chain comprising a human H chain C region, and an H chain V region of mouse monoclonal antibody against human IL-8.

8. Chimeric H chain as set forth in claim 7, wherein said mouse H chain V-region has the amino acid sequence or a portion thereof shown in SEQ ID NO: 27.
9. Chimeric antibody comprising:
 - (1) L chains each comprising a human L chain constant region (C region), and an L chain V region of mouse monoclonal antibody against human IL-8; and,
 - (2) H chains each comprising a human H chain C region, and an H chain V region of mouse monoclonal antibody against human IL-8.
10. Chimeric antibody as set forth in claim 9 wherein said mouse L chain V region has the amino acid sequence or a portion thereof shown in SEQ ID NO: 26, and said mouse H chain V region has the amino acid sequence or a portion thereof shown in SEQ ID NO: 27.
11. A complementarity determining region (CDR) of an L chain V region of mouse monoclonal antibody against human IL-8.
12. A CDR as set forth in claim 11 having the amino acid sequence or a portion thereof shown below and in SEQ ID NO: 26.
- CDR1: Arg Ala Ser/Glu Ile Ile/Tyr Ser Tyr/Leu Ala/
 CDR2: Asn Ala Lys/Thr Leu Ala/Asp
 CDR3: Gln His His/Phe Gly Phe/Pro Arg Thr/
13. A CDR of an H chain V region of mouse monoclonal antibody against human IL-8.
14. A CDR as set forth in claim 13 having the amino acid sequence or a portion thereof shown below and in SEQ ID NO: 27.
- CDR1: Asp Tyr Tyr/Leu Ser
 CDR2: Leu Ile Arg/Asn Lys Ala/Asn Gly Tyr/Thr Arg Glu/Tyr Ser Ala/Ser Val Lys/Gly
 CDR3: Glu Asn Tyr/Arg Tyr Asp/Val Glu Leu/Ala Tyr/
15. A reshaped human L chain V region of antibody against human IL-8 comprising:
 - (1) a framework region (FR) of a human L chain V region; and,
 - (2) a CDR of an L chain V region of mouse monoclonal antibody against human IL-8.
16. A reshaped human L chain V region as set forth in claim 15, wherein said CDR has the amino acid sequence or a portion thereof shown in claim 12.
17. A reshaped human L chain V region as set forth in claims 15 and 16, wherein said FR is derived from human antibody RE1.
18. A reshaped human L chain V region as set forth in claim 15, wherein said L chain V region has the amino acid sequence or a portion thereof indicated as RVL_a or RVL_b in Table 2.
19. A reshaped human H chain V region of antibody against human IL-8 comprising:
 - (1) an FR of a human H chain V region; and,
 - (2) a CDR of an H chain V region of mouse monoclonal antibody against human IL-8.
20. A reshaped human H chain V region as set forth in claim 19, wherein said CDR has the amino acid sequence or a portion thereof shown in claim 14.
21. A reshaped human H chain V region as set forth in claims 19 and 20, wherein said FR1, FR2 and FR3 are derived from human antibody VDH26 and said FR4 is derived from human antibody 4B4.
22. A reshaped human H chain V region as set forth in claim 19, wherein said H chain V region has the amino acid

sequence or a portion thereof indicated as RVHa, RVHb, RVHc, RVHd, RVHe, RVHf, RVHg or RVHh in Tables 3 and 4.

23. An L chain of reshaped human antibody against human IL-8 comprising:

- (1) a human L chain C region; and,
- (2) an L chain V region comprising a human L chain FR, and an L chain CDR of mouse monoclonal antibody against human IL-8.

10 24. An L chain of reshaped human antibody as set forth in claim 23, wherein said human L chain C region is a human C_k region, human L chain FR is derived from human antibody RE1, and said L chain CDR has the amino acid sequence or a portion thereof shown in claim 12.

15 25. An L chain of reshaped human antibody as set forth in claim 23, wherein said L chain V region has the amino acid sequence or a portion thereof indicated as RVL_a or RVL_b in Table 2.

26. An H chain of reshaped human antibody against human IL-8 comprising:

- (1) a human H chain C region; and,
- (2) an H chain V region comprising a human H chain FR, and an H chain CDR of mouse monoclonal antibody against human IL-8.

20 27. An H chain of reshaped human antibody as set forth in claim 26, wherein said human H chain C region is a human C_{y1} region, said human H chain FR1, FR2 and FR3 are derived from human antibody VDH26, human H chain FR4 is derived from human antibody 4B4, and said H chain CDR has the amino acid sequence or a portion thereof shown in claim 14.

25 28. An H chain of reshaped human antibody as set forth in claim 26, wherein said H chain V region has the amino acid sequence or a portion thereof indicated as RVHa, RVHb, RVHc, RVHd, RVHe, RVHf, RVHg and RVHh Tables 3 and 4.

30 29. A reshaped human antibody against human IL-8 comprising:

(A) L chains each comprising:

- (1) a human L chain C region; and,
- (2) an L chain V region comprising a human

35 L chain FR, and L chain CDR of mouse monoclonal antibody against human IL-8; and,

(B) an H chain comprising:

- (1) a human H chain C region; and,
- (2) an H chain V region comprising a human H chain FR, and H chain CDR of mouse monoclonal antibody against human IL-8.

40 30. A reshaped human antibody as set forth in claim 29, wherein said L chain CDR has the amino acid sequence or a portion thereof shown in claim 12, and said H chain CDR has the amino acid sequence or a portion thereof shown in claim 14.

45 31. A reshaped human antibody as set forth in claim 29, wherein said L chain CDR has the amino acid sequence or a portion thereof shown in claim 12, and said H chain CDR has the amino acid sequence or a portion thereof shown in claim 14; said human L chain FR is derived from human antibody RE1; said human H chain FR1, FR2 and FR3 are derived from human antibody VDH26, human H chain FR4 is derived from human antibody 4B4, and said human L chain C region is a human C_k region; and, said human H chain C region is a human C_{y1} region.

50 32. A reshaped human antibody as set forth in claim 29, wherein said L chain CDR has the amino acid sequence or a portion thereof shown in claim 12, and said H chain CDR has the amino acid sequence or a portion thereof shown in claim 14; said human L chain FR is derived from human antibody RE1; said human H chain FR1, FR2 and FR3 are derived from human antibody VDH26, human H chain FR4 is derived from human antibody 4B4, and said human

L chain C region is a human C_k region; and, said human H chain C region is C_{γ4}.

33. A reshaped human antibody as set forth in claim 29 wherein said L chain V region has the amino acid sequence or a portion thereof indicated as RVL_a or RVL_b in Table 2.

5 34. A reshaped human antibody as set forth in claim 29, wherein said H chain V region has the amino acid sequence or a portion thereof indicated as RVH_a, RVH_b, RVH_c, RVH_d, RVH_e, RVH_f, RVH_g or RVH_h in Tables 3 and 4.

10 35. DNA that codes for a chimeric L chain of antibody against human IL-8 containing:
(1) a human L chain C region; and,
(2) an L chain V region of mouse monoclonal antibody against human IL-8.

15 36. DNA as set forth in claim 35, wherein said L chain V region codes for the amino acid sequence or a portion thereof shown in SEQ ID NO: 26.

37. DNA as set forth in claim 35, wherein the DNA that codes for said L chain V region has the nucleotide sequence or a portion thereof shown in SEQ ID NO: 26.

20 38. DNA that codes for a chimeric H chain of antibody against human IL-8 containing:
(1) a human H chain C region; and,
(2) an H chain V region of mouse monoclonal antibody against human IL-8.

25 39. DNA as set forth in claim 38, wherein said H chain V region has the amino acid sequence or a portion thereof shown in SEQ ID NO: 27.

40. DNA as set forth in claim 38, wherein said H chain V region has the nucleotide sequence or a portion thereof shown in SEQ ID NO: 27.

30 41. DNA that codes for an L chain V region of mouse monoclonal antibody against human IL-8.

42. DNA as set forth in claim 41, wherein said L chain V region codes for the amino acid sequence or a portion thereof shown in SEQ ID NO: 26.

35 43. DNA as set forth in claim 41, wherein the DNA that codes for said L chain V region has the nucleotide sequence or a portion thereof shown in SEQ ID NO: 26.

44. DNA that codes for an H chain V region of mouse monoclonal antibody against human IL-8.

40 45. DNA as set forth in claim 44, wherein said H chain V region codes for the amino acid sequence or a portion thereof shown in SEQ ID NO: 27.

46. DNA as set forth in claim 44, wherein the DNA that codes for said H chain V region has the nucleotide sequence or a portion thereof shown in SEQ ID NO: 27.

45 47. DNA that codes for CDR of an L chain V region of mouse monoclonal antibody against human IL-8.

48. DNA that codes for CDR as set forth in claim 47, wherein said CDR codes for the amino acid sequence or a portion thereof shown in claim 12.

50 49. DNA that codes for CDR as set forth in claim 47, wherein said CDR has the nucleotide sequence or a portion thereof shown in SEQ ID NO: 26.

55 50. DNA that codes for CDR of an H chain V region of mouse monoclonal antibody against human IL-8.

51. DNA that codes for CDR as set forth in claim 50, wherein said CDR has the amino acid sequence or a portion thereof shown in claim 14.

52. DNA that codes for CDR as set forth in claim 50, wherein said CDR has the nucleotide sequence or a portion thereof shown in SEQ ID NO: 27.

53. DNA that codes for a reshaped human L chain V region of antibody against human IL-8 comprising:

5 (1) FR of a human L chain V region; and,
 (2) CDR of an L chain V region of mouse monoclonal antibody against human IL-8.

54. DNA that codes for a reshaped human L chain V region as set forth in claim 53, wherein said CDR has the amino acid sequence or a portion thereof shown in claim 12.

10 55. DNA that codes for a reshaped human L chain V region as set forth in claims 53 and 54, wherein said FR is derived from human antibody REI.

15 56. DNA as set forth in claim 53, wherein said L chain V region codes for the amino acid sequence or a portion thereof indicated as RVL_a or RVL_b in Table 2.

57. DNA as set forth in claim 53 having the nucleotide sequence of a portion thereof shown in SEQ ID NO: 62 or SEQ ID NO: 65.

20 58. DNA that codes for a reshaped human H chain V region of antibody against human IL-8 comprising:

25 (1) FR of a human H chain V region; and,
 (2) CDR of an H chain V region of mouse monoclonal antibody against human IL-8.

59. DNA that codes for a reshaped human H chain V region as set forth in claim 58, wherein said CDR has the amino acid sequence or a portion thereof shown in claim 14.

30 60. DNA that codes for a reshaped human H chain V region as set forth in claims 58 and 59, wherein said FR1, FR2 and FR3 is derived from human antibody VDH26, and FR4 are derived from human antibody 4B4.

61. DNA that codes for a reshaped human H chain V region as set forth in claim 58, wherein said H chain V region codes for the amino acid sequence or a portion thereof indicated as RVHa, RVHb, RVHc, RVHd, RVHe, RVHf, RVHg or RVHh in Tables 3 and 4.

35 62. DNA as set forth in claim 48 having the nucleotide sequence or a portion thereof shown in SEQ ID NO: 38, 41, 44, 45, 48, 51, 54 or 55.

63. DNA that codes for a reshaped human L chain of antibody against human IL-8 comprising:

40 (1) a human L chain C region; and,
 (2) an L chain V region comprising human FR, and CDR of mouse monoclonal antibody against human IL-8.

64. DNA as set forth in claim 63, wherein said L chain V region codes for the amino acid sequence or a portion thereof indicated as RVL_a or RVL_b in Table 2.

45 65. DNA as set forth in claim 63, wherein said L chain V region has the nucleotide sequence or a portion thereof shown in SEQ ID NO: 62 or SEQ ID NO: 65.

50 66. DNA as set forth in claims 63, 64 and 65, wherein said human L chain C region is a human L chain C_x region.

67. DNA that codes for a reshaped human H chain of antibody against human IL-8 comprising:

55 (1) a human H chain C region; and,
 (2) an H chain V region comprising human FR, and CDR of mouse monoclonal antibody against human IL-8.

68. DNA that codes for reshaped human H chain as set forth in claim 67 wherein said H chain V region codes for the amino acid sequence or a portion thereof indicated as RVHa, RVHb, RVHc, RVHd, RVHe, RVHf, RVHg or RVHh in Tables 3 and 4.

69. DNA as set forth in claim 67, wherein said H chain V region has the nucleotide sequence or a portion thereof shown in SEQ ID NO: 38, 41, 44, 45, 48, 51, 54 or 55.

5 70. DNA as set forth in claims 67, 68 or 69, wherein said human H chain C region is a human H chain C_{y1} region.

71. DNA as set forth in claims 67, 68 or 69, wherein said human H chain C region is a human H chain C_{y4} region.

10 72. A vector containing DNA as set forth in any one of claims 35, 36, 37, 38, 39, 40, 63, 64, 65, 66, 67, 68, 69, 70 and 71.

15 73. A host cell transformed by a vector as set forth in claim 72.

74. A process for producing chimeric antibody against human IL-8 comprising the steps of culturing host cells transformed simultaneously with an expression vector containing DNA as set forth in any one of claims 35, 36 and 37, and an expression vector containing DNA as set forth in any one of claims 38, 39 and 40, and recovering the target antibody.

20 75. A process for producing chimeric antibody against human IL-8 comprising the steps of culturing host cells transformed with an expression vector containing DNA as set forth in any one of claims 35, 36 and 37, and DNA as set forth in any one of claims 38, 39 and 40, and recovering the target antibody.

25 76. A process for producing reshaped human antibody against human IL-8 comprising the steps of culturing host cells transformed simultaneously with an expression vector containing DNA as set forth in any one of claims 63, 64, 65 and 66, and an expression vector containing DNA as set forth in any one of claims 67, 68, 69, 70 and 71, and recovering the target antibody.

77. A process for producing reshaped human antibody against human IL-8 comprising the steps of culturing host cells transformed with an expression vector containing DNA as set forth in any one of claims 63, 64, 65 and 66, and DNA as set forth in any one of claims 67, 68, 69, 70 and 71, and recovering the target antibody.

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Fig. 1

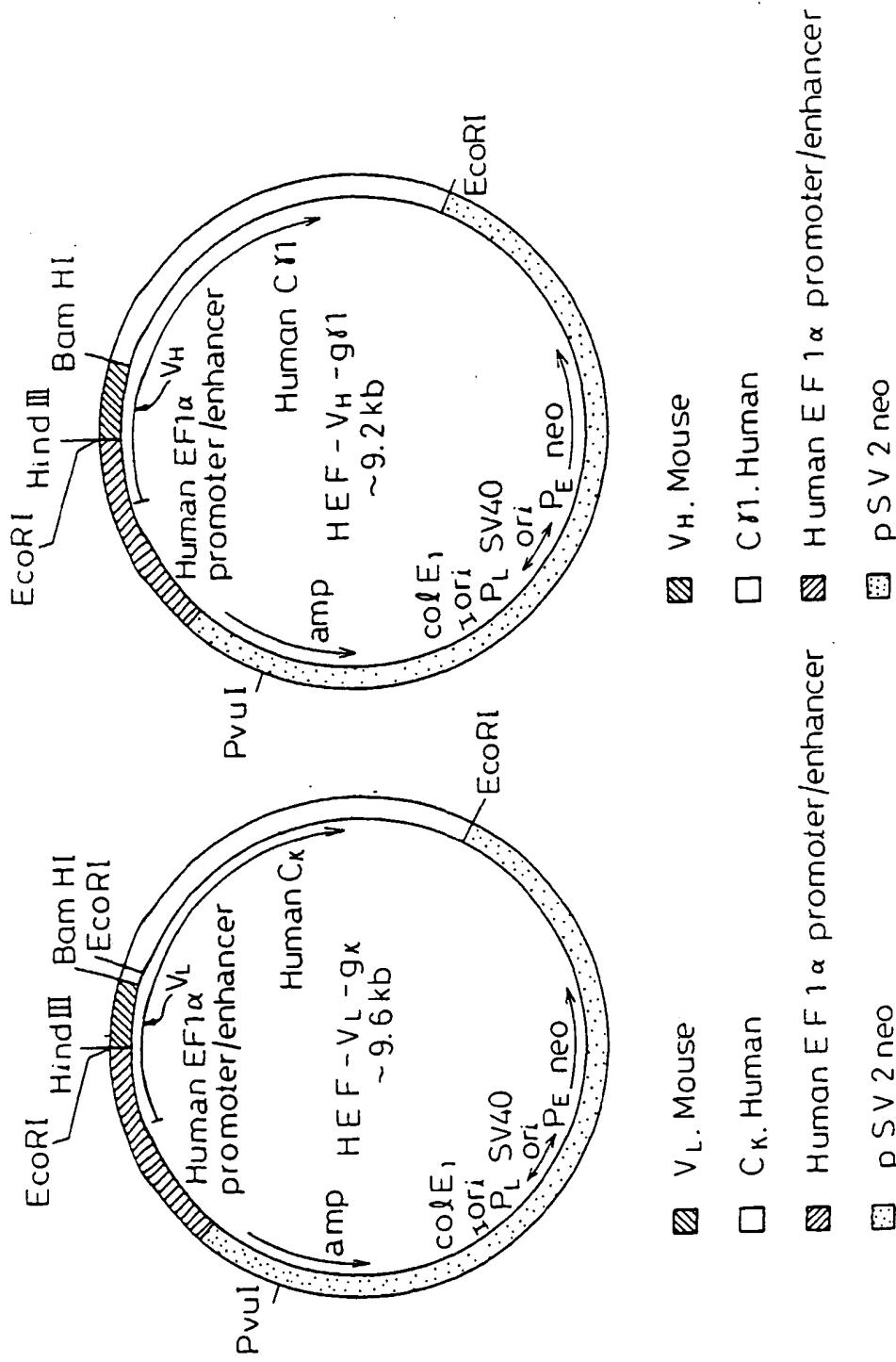


Fig. 2

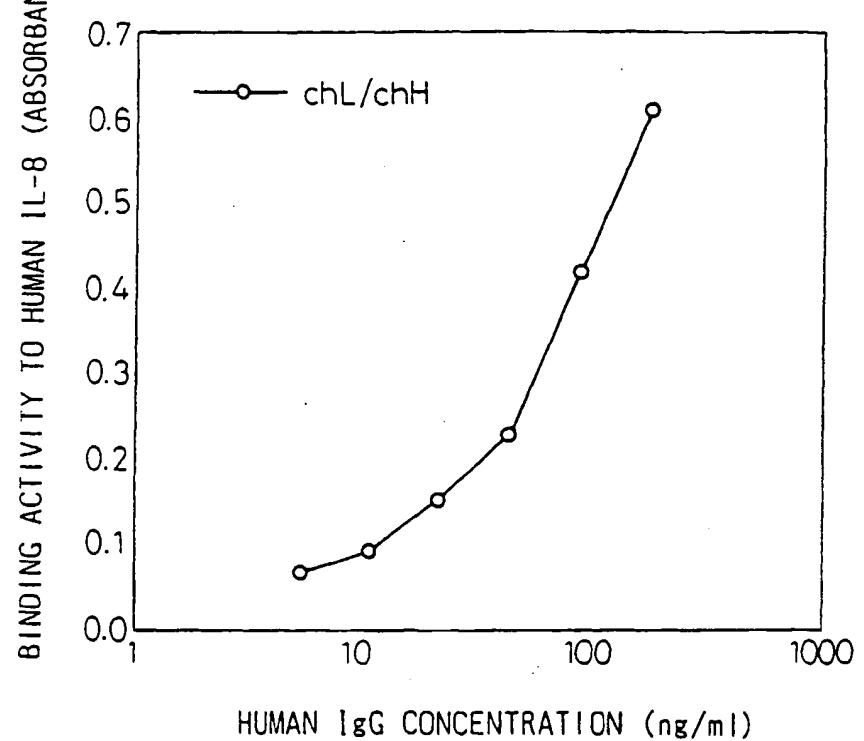
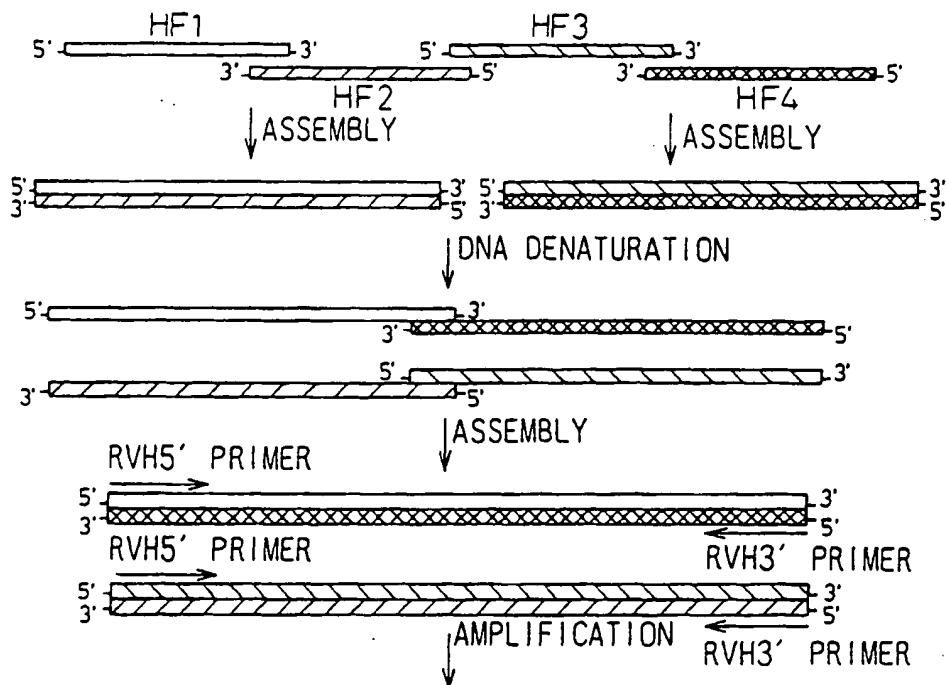


Fig. 3

A

OLIGONUCLEOTIDE SYNTHESIS



B

OLIGONUCLEOTIDE SYNTHESIS

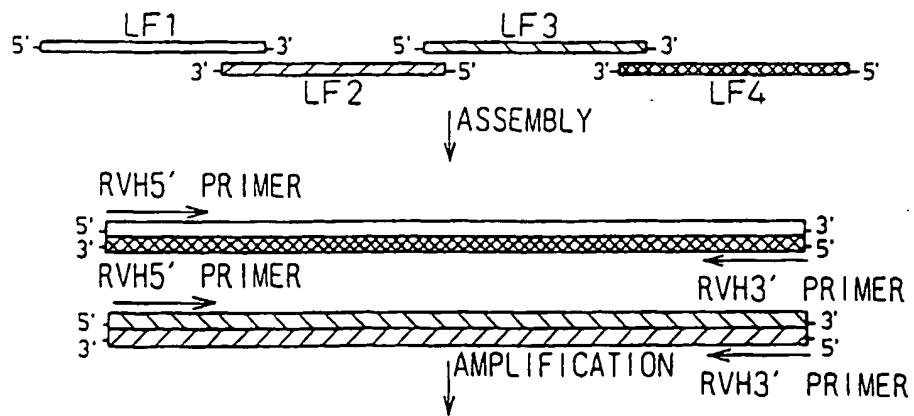


Fig.4

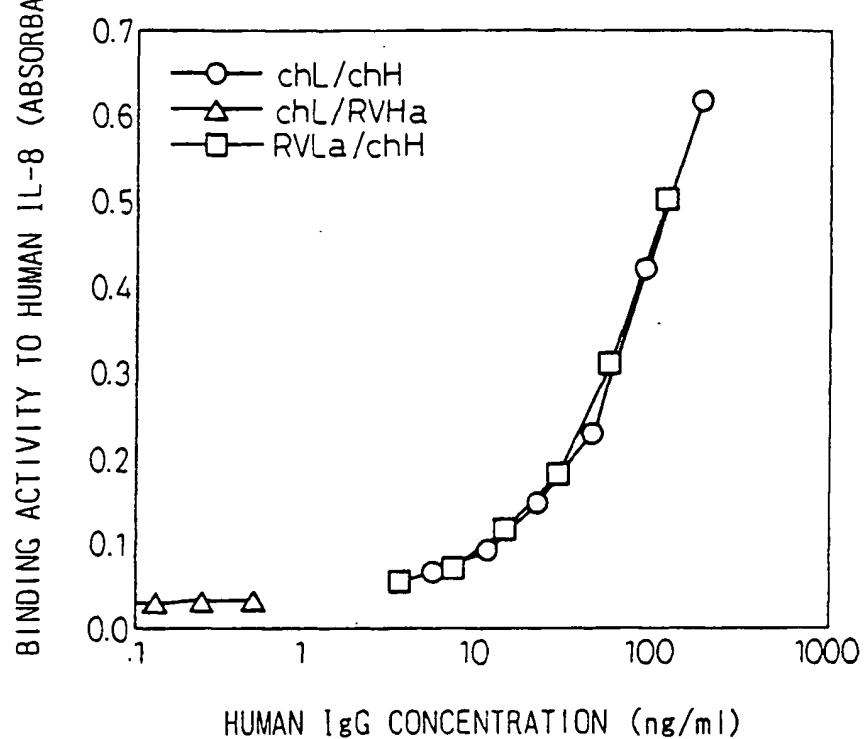


Fig.5

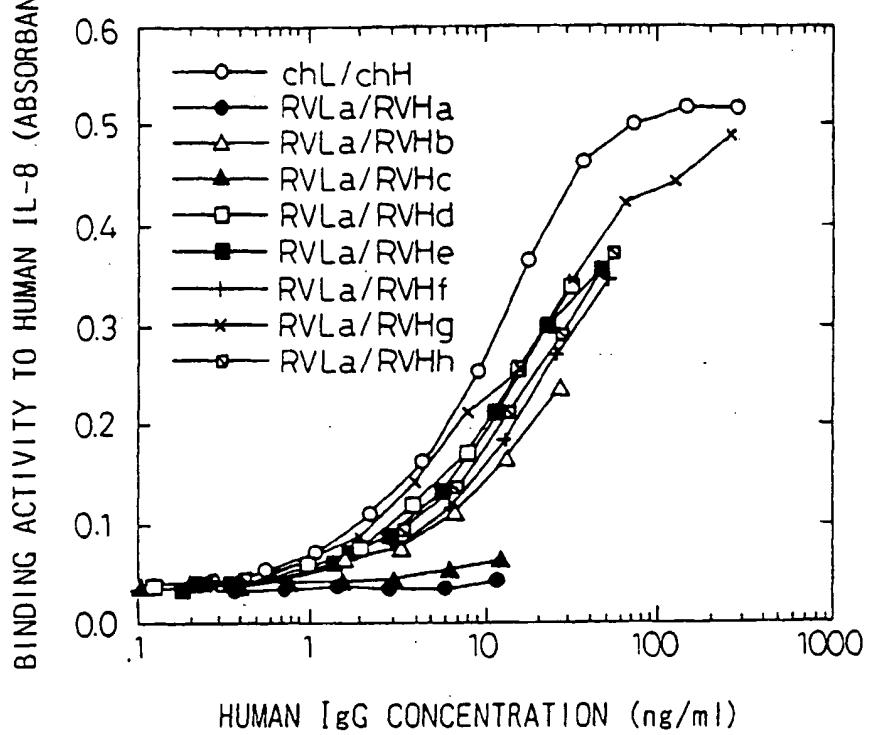


Fig.6

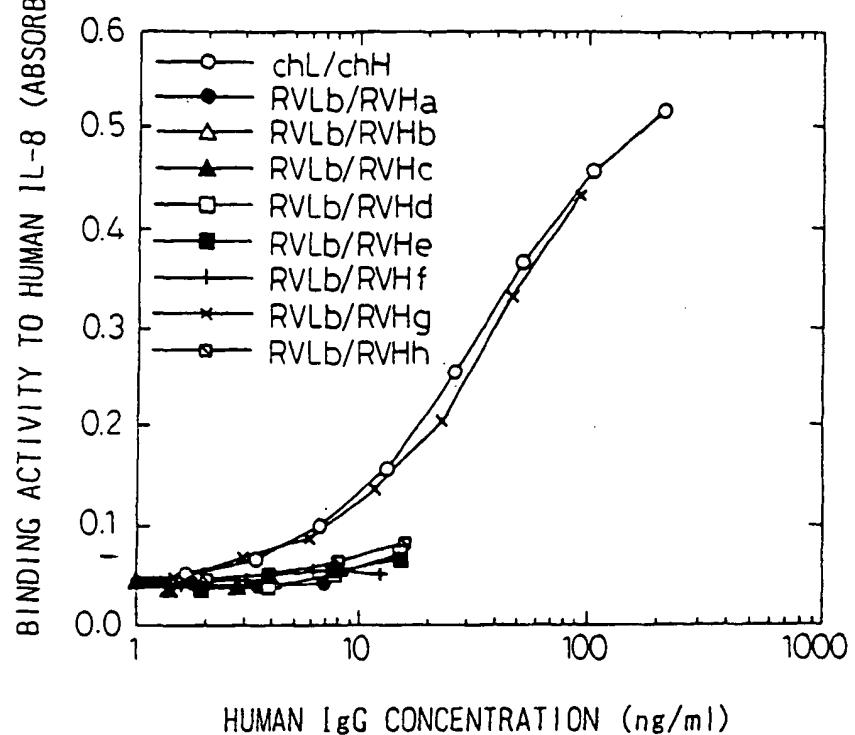


Fig.7

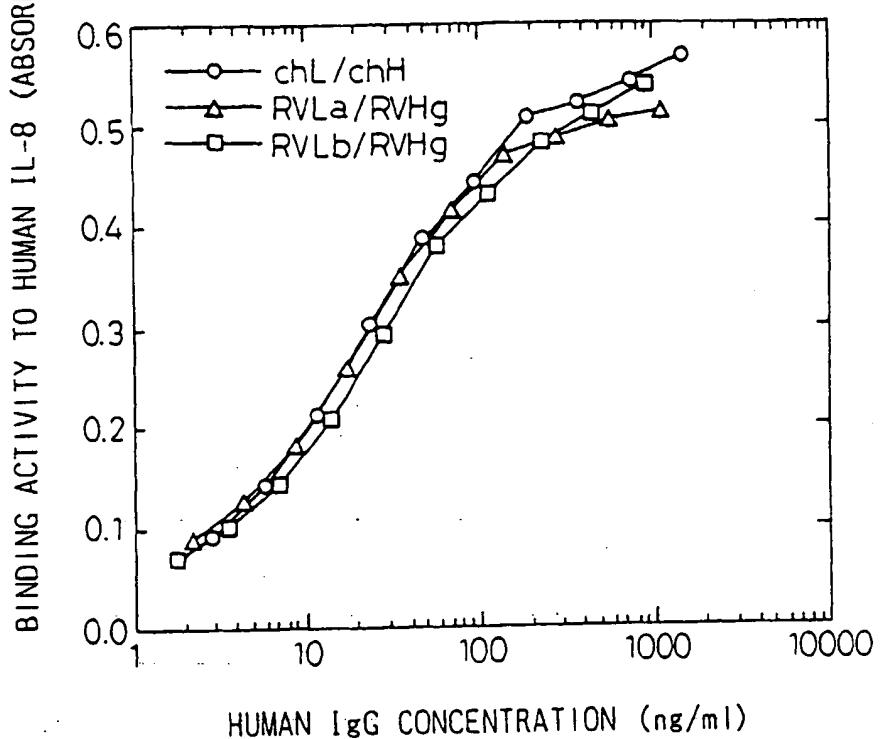
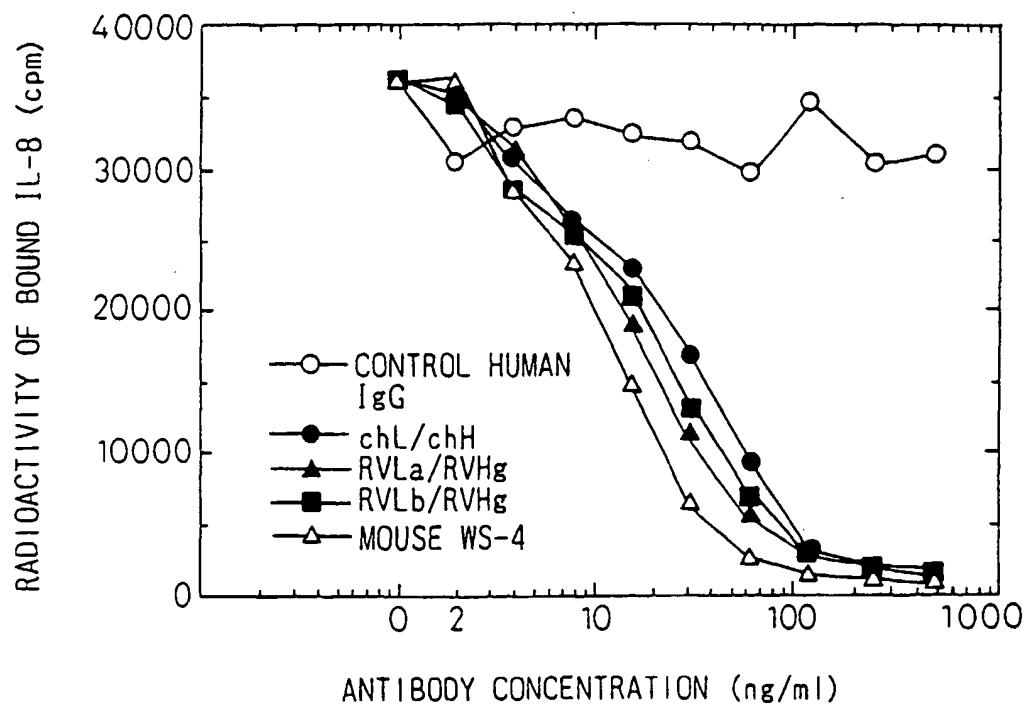


Fig.8



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP95/01396

A. CLASSIFICATION OF SUBJECT MATTER
 Int. C16 C07K16/24, C12N15/13, 15/62, C12P21/02, 21/08,
 C12N1/21// (C12P21/02, C12R1:19)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. C16 C12N15/02-15/90, C12P21/00, 21/02, 21/08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS PREVIEWS, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KO, Yue-chau, et al. "A sensitive enzyme-linked immunosorbent assay for human interleukin-8", J. Immunol. Methods, 1992, Vol. 149, p. 227-235	1-20, 23-26, 29, 30, 33, 35-59, 62-67, 70-77
Y	Osamu Kanemitsu, "Antibody Engineering" 1st edit. (Tokyo), Chijin Shokan K.K. (25. 01. 1995), p. 195-234	1-20, 23-26, 29, 30, 33, 35-59, 62-67, 70-77
Y	RIECHMANN, L., et al. "Reshaping human antibodies for therapy", Nature, 1988, Vol. 332, p. 323-327	1-20, 23-26, 29, 30, 33, 35-59, 62-67, 70-77
A	BULUWELA, L., et al. "The use of chromosomal translocations to study human immunoglobulin gene organization : mapping D _H segments within 35kb of the Cu gene and identification of a new D _H locus", The EMBO Journal, 1988, Vol. 7, No. 7, p. 2003-2010	21, 22, 27, 28, 31, 32, 34, 60, 61, 68, 69

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

October 9, 1995 (09. 10. 95)

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP95/01396

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SANZ, I., et al. "V _H sequence of a human anti-Sm autoantibody", J. Immunol., 1989, Vol. 142, No. 3, p. 883-887	21, 22, 27, 28, 31, 32, 34, 60, 61, 68, 69

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